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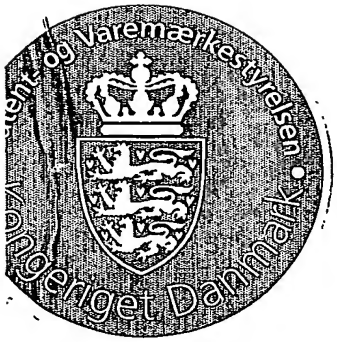

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PATENT- OG VAREMÆRKESTYRELSEN

Templated molecules and methods for using such molecules

Technical Field of the Invention

Biological systems allow template-directed synthesis of α-peptides. The present invention enables a system that allows template-directed synthesis of other types of polymers as well as α-peptides. The present invention relates to templated molecules and templated molecules linked to a predetermined template. The templated molecules comprise a sequence of functional groups that are linked together. Each functional group is initially linked to an element capable of complementing a predetermined coding element of the template. Following complementation of a coding element, or complementation of a plurality of coding elements, the appended functional groups are linked and the templated moleculed is formed.

Background

The central dogma in biology describes the flow of information as a one-way process from DNA to RNA to polypeptide. Accordingly, DNA is transcribed by a RNA polymerase into mRNA; and the mRNA is subsequently then translated into protein by the ribosomes and tRNAs.

The direct relation between the DNA and the protein, i.e., the fact that the sequence of triplet codons defines the sequence of α-amino acid residues in a polypeptide, has allowed the development of numerous molecular biological methods, in which the experimenter manipulates the DNA (mutagenizes, recombines, deletes, inserts, etc), and then uses in vivo systems (e.g., microbes) or in vitro systems (e.g., Zubay in vitro expression systems) to transfer the resulting changes from the DNA level to the level of the templated polypeptide, i.e., to mutate, recombine, delete, insert, etc. the polypeptide.

Several systems have been invented that allows a flow of information from polypeptide to DNA. These systems are phage display, ribosome/polysome display, peptides-on-plasmid display, and other systems. These systems introduce a physical link between the template (e.g., DNA) and the templated molecule (polypeptide). As a result, it is possible, from a population of templated molecules linked to the tem-

plate that templated the synthesis of the molecule, to first enrich for a desired characteristic of the templated molecule (e.g., binding of the templated molecule to an affinity column), and then amplify the enriched population of templated molecules through amplification of its template (DNA or RNA), followed by translation of the amplified templates. These methods have been used to identify polypeptides with novel and/or improved features from libraries consisting of from a million to about 10^{15} polypeptides.

The critical feature of the prior art systems is the amplifiability of the templated molecule, through amplification of its template. Thus, after the selection step in which molecules with the desired property are enriched, the enriched population may be amplified and then taken through yet a selection step, etc. - the process of selection-and-amplification may be repeated many times. In this way the "noise" of the selection assay is averaged out over several selection-and-amplification rounds, and even if the individual selection step only enriches e.g. 10-fold, a theoretical enrichment of 10^{12} can be reached after 12 selection-and-amplification rounds. Had the molecules *not* been amplifiable, the same enrichment would have had to be achieved in a single screening step, which means that the enrichment in this one step would have had to be 10^{12}, and the assay should still have the same overall stringency (accuracy). This is practically impossible with current technologies.

In the field of chemistry, a different combinatorial approach has been developed. This approach involved the parallel synthesis of millions of related compounds, in an array (where each position defined a specific compound), or on beads (where one bead carried many copies of the same compound). The population of compounds were then screened for desired characteristics. Importantly, this type of combinatorial library has no means for amplification, and therefore requires the use of very stringent screening methods, as explained above. Recently, the trend in for example medicinal chemistry has therefore been to use less diverse, but better characterized libraries.

Principles for tagging chemical libraries have also been developed. For example, systems that employed DNA oligos to tag molecule libraries have been developed as exemplified herein below. The tag is used as a means of identification, but can-

not be used to template the synthesis of the tagged molecule. Therefore, despite the tag, these systems still require a very efficient screening method.

The below listed references illustrate some of the above-mentioned short-comings of the prior art methods in the field of the invention.

EP 0 604 552 B1 relates to a method for synthesizing diverse collections of oligomers. The invention involves the use of an identifier tag to identify the sequence of monomers in an oligomer. The identifier tags facilitate subsequent identification of reactions through which members of a library of different synthetic compounds have been synthesised in a component by component fashion.

EP 0 643 778 B1 relates to encoded combinatorial chemical libraries. Each of a collection of polypeptides is labelled by an appended "genetic" tag, itself constructed by chemical synthesis, to provide a "retro-genetic" way of specifying each polypeptide.

EP 0 773 227 A1 relates to a method for preparing a new pharmaceutical drug or diagnostic reagent, which includes the step of screening, against a ligand or receptor, a library of different synthetic compounds obtainable by synthesis in a component by component fashion.

US 4,863,857 relates to a method for determining the amino acid sequence of a polypeptide complementary to at least a portion of an original peptide or protein. In one aspect the method involves: (a) determining a first nucleotide sequence of a first nucleic acid coding for the biosynthesis of at least a portion of the original peptide or protein; (b) ascertaining a second nucleotide sequence of a second nucleic acid which base-pairs with the first nucleotide sequence of the first nucleic acid, the first and second nucleic acids pairing in antiparallel directions; and (c) determining the amino acid sequence of the complementary polypeptide by the second nucleotide sequence when read in the same reading frame as the first nucleotide sequence.

US 5,162,218 relates to polypeptide compositions having a binding site specific for a particular target ligand and further having an active functionality proximate the binding site. The active functionality may be a reporter molecule, in which case the

polypeptide compositions are useful in performing assays for the target ligand. Also disclosed are methods for preparing polypeptides having active functionalities proximate their binding site, said method comprising the step of combining the polypeptide specific for the target ligand with an affinity label having a reactive group attached thereto. The reactive group is then covalently attached to an amino acid side chain proximate the binding site and cleaved from the substrate. The substrate is subsequently eluted, leaving a moiety of the reactive group covalently attached to the polypeptide. The active funtionality may then be attached to the moiety.

US 5,270,170 relates to a random peptide library constructed by transforming host cells with a collection of recombinant vectors that encode a fusion protein comprised of a DNA binding protein and a random peptide and also encode a binding site for the DNA binding protein. The fusion protein can be used for screening ligands. The screening method results in the formation of a complex comprising the fusion protein bound to a receptor through the random peptide ligand and to the recombinant DNA vector through the DNA binding protein.

US 5,539,082 relates to a novel class of compounds, known as peptide nucleic acids capable of binding complementary ssDNA and RNA strands more strongly than a corresponding DNA. The peptide nucleic acids generally comprise ligands such as naturally occurring DNA bases attached to a peptide backbone through a suitable linker.

US 5,574,141 relates to functionalized carrier materials for the simultaneous synthesis and direct labeling of oligonucleotides as primers for template-dependent enzymatic nucleic acid syntheses. The polymeric carriers are loaded with nucleic acid building blocks which in turn contain labelling groups or precursors thereof. The polymeric carrier loaded in this way serves as a solid or liquid phase for the assembly of oligonucleotides which can be used as primers for a template-dependent enzymatic nucleic acid synthesis such as in sequencing analysis or in the polymerase chain reaction (PCR).

US 5,573,905 relates to an encoded combinatorial chemical library comprised of a plurality of bifunctional molecules having both a chemical polymer and an identifier oligonucleotide sequence that defines the structure of the chemical polymer. Also

described are the bifunctional molecules of the library, and methods of using the library to identify chemical structures within the library that bind to biologically active molecules in preselected binding interactions.

US 5,597,697 relates to a screening assay for inhibitors and activators of RNA and DNA-dependent nucleic acid polymerases. The invention provides methods for the identification and discovery of agents which are inhibitors and activators of RNA and DNA-dependent nucleic acid polymerases. The essential feature of the invention is the incorporation of a functional polymerase binding site sequence (PBS) into a nucleic acid molecule which is chosen for its ability to confer a discernible characteristic via its sequence specific activity such that the incorporation of the PBS renders the nucleic acid molecule a functional template for a predetermined RNA or DNA-template directed nucleic acid polymerase. In the presence of the polymerase, suitable primer molecules, and any necessary accessory molecules, catalytic extension of the strand of nucleic acids complementary to the template occurs, resulting in a partial or total elimination of (or increase in) the characteristic conferring activity of the reporter-template molecule described due to the antisense effects of the complementary strand or other polymerase-mediated effects.

US 5,639,603 relates to a method for synthesizing and screening molecular diversity by means of a general stochastic method for synthesizing compounds. The method can be used to generate large collections of tagged compounds that can be screened to identify and isolate compounds with useful properties.

US 5,698,685 relates to a morpholino-subunit combinatorial library and a method for generating a compound capable of interacting specifically with a selected macromolecular ligand. The method involves contacting the ligand with a combinatorial library of oligomers composed of morpholino subunits with a variety of nucleobase and non-nucleobase side chains. Oligomer molecules that bind specifically to the receptor are isolated and their sequence of base moieties is determined. Also disclosed is a combinatorial library of oligomers useful in the method and novel morpholino-subunit polymer compositions.

US 5,708,153 relates to a method for synthesizing diverse collections of tagged compounds by means of a general stochastic method for synthesizing random oli-

gomers on particles. A further aspect of the invention relates to the use of identification tags on the particles to facilitate identification of the sequence of the monomers in the oligomer.

US 5,719,262 relates to a novel class of compounds, known as peptide nucleic acids, which bind complementary DNA and RNA strands more strongly than the corresponding DNA or RNA strands, and exhibit increased sequence specificity and solubility. The peptide nucleic acids comprise ligands selected from a group consisting of naturally-occurring nucleobases and non-naturally-occurring nucleobases attached to a polyamide backbone, and contain alkylamine side chains.

US 5,721,099 relates to encoded combinatorial chemical libraries encoded with tags. Encoded combinatorial chemistry is provided, whereby sequential synthetic schemes are recorded using organic molecules, which define choice of reactant, and stage, as the same or different bit of information. Various products can be produced in the multi-stage synthesis, such as oligomers and synthetic non-repetitive organic molecules. Particularly, pluralities of identifiers may be used to provide a binary or higher code, so as to define a plurality of choices with only a few detachable tags. The particles may be screened for a characteristic of interest, particularly binding affinity, where the products may be detached from the particle or retained on the particle. The reaction history of the particles which are positive for the characteristic can be determined by the release of the tags and analysis to define the reaction history of the particle.

US 5,723,598 relates to an encoded combinatorial chemical library comprised of a plurality of bifunctional molecules having both a chemical polymer and an identifier oligonucleotide sequence that defines the structure of the chemical polymer. Also described are the bifunctional molecules of the library, and methods of using the library to identify chemical structures within the library that bind to biologically active molecules in preselected binding interactions.

US 5,770,358 relates to tagged synthetic oligomer libraries and a general stochastic method for synthesizing random oligomers. The method can be used to synthesize compounds to screen for desired properties. The use of identification tags on the oligomers facilitates identification of oligomers with desired properties.

US 5,786,461 relates to peptide nucleic acids having amino acid side chains. A novel class of compounds, known as peptide nucleic acids, bind complementary DNA and RNA strands more strongly than the corresponding DNA or RNA strands, and exhibit increased sequence specificity and solubility. The peptide nucleic acids comprise ligands selected from a group consisting of naturally-occurring nucleobases and non-naturally-occurring nucleobases attached to a polyamide backbone, and contain alkylamine side chains.

US 5,789,162 relates to a method for synthesizing diverse collections of oligomers. A general stochastic method for synthesizing random oligomers on particles is disclosed. A further aspect of the invention relates to the use of identification tags on the particles to facilitate identification of the sequence of the monomers in the oligomer.

US 5,840,485 relates to topologically segregated, encoded solid phase libraries. Libraries of synthetic test compounds are attached to separate phase synthesis supports that also contain coding molecules that encode the structure of the synthetic test compound. The molecules may be polymers or multiple nonpolymeric molecules. The synthetic test compound can have backbone structures with linkages such as amide, urea, carbamate (i.e., urethane), ester, amino, sulfide, disulfide, or carbon-carbon, such as alkane and alkene, or any combination thereof. The synthetic test compound can also be molecular scaffolds, or other structures capable of acting as a scaffolding. The invention also relates to methods of synthesizing such libraries and the use of such libraries to identify and characterize molecules of interest from among the library of synthetic test compounds.

US 5,843,701 relates to systematic polypeptide evolution by reverse translation and a method for preparing polypeptide ligands of target molecules wherein candidate mixtures comprised of ribosome complexes or mRNA:polypeptide copolymers are partitioned relative to their affinity to the target and amplified to create a new candidate mixture enriched in ribosome complexes or mRNA:polypeptide copolymers with an affinity to the target.

US 5,846,839 relates to a method for hard-tagging an encoded synthetic library. Disclosed are chemical encryption methods for determining the structure of compounds formed in situ on solid supports by the use of specific amine tags which, after compound synthesis, can be deencrypted to provide the structure of the compound found on the support.

US 5,922,545 relates to methods and compositions for identifying peptides and single-chain antibodies that bind to predetermined receptors or epitopes. Such peptides and antibodies are identified by methods for affinity screening of polysomes displaying nascent peptides.

US 5,958,703 relates to methods for screening libraries of complexes for compounds having a desired property such as the capacity to bind to a cellular receptor. The complexes in such libraries comprise a compound under test, a tag recording at least one step in synthesis of the compound, and a tether susceptible to modification by a reporter molecule. Modification of the tether is used to signify that a complex contains a compound having a desired property. The tag can be decoded to reveal at least one step in the synthesis of such a compound

US 5,986,053 relates peptide nucleic acid complexes of two peptide nucleic acid strands and one nucleic acid strand. Peptide nucleic acids and analogues of peptide nucleic acids are used to form duplex, triplex, and other structures with nucleic acids and to modify nucleic acids. The peptide nucleic acids and analogues thereof also are used to modulate protein activity through, for example, transcription arrest, transcription initiation, and site specific cleavage of nucleic acids.

US 5,998,140 relates to methods and compositions for forming complexes intracellularly between dsDNA and oligomers of heterocycles, aliphatic amino acids, particularly omega-amino acids, and a polar end group. By appropriate choice of target sequences and composition of the oligomers, complexes are obtained with low dissociation constants.

US 6,060,596 relates to an an encoded combinatorial chemical library comprised of a plurality of bifunctional molecules having both a chemical polymer and an identifier oligonucleotide sequence that defines the structure of the chemical polymer. Also

described are the bifunctional molecules of the library, and methods of using the library to identify chemical structures within the library that bind to biologically active molecules in preselected binding interactions.

US 6,080,826 relates to Template-directed ring-closing metathesis and ring-opening metathesis polymerization of functionalized dienes. Functionalized cyclic olefins and methods for making the same are disclosed. Methods include template-directed ring-closing metathesis ("RCM") of functionalized acyclic dienes and template-directed depolymerization of functionalized polymers possessing regularly spaced sites of unsaturation. Although the template species may be any anion, cation, or dipolar compound, cationic species, especially alkali metals, are preferred. Functionalized polymers with regularly spaced sites of unsaturation and methods for making the same are also disclosed. One method for synthesizing these polymers is by ring-opening metathesis polymerization ("ROMP") of functionalized cyclic olefins.

US 6,127,154 relates to compounds which possess a complementary structure to a desired molecule, such as a biomolecule, in particular polymeric or oligomeric compounds, which are useful as in vivo or in vitro diagnostic and therapeutic agents are provided. Also, various methods for producing such compounds are provided.

US 6,140,493 relates to a method for synthesizing diverse collections of oligomers. A general stochastic method for synthesizing random oligomers is disclosed and can be used to synthesize compounds to screen for desired properties. Identification tags on the oligomers facilitates identification of oligomers with desired properties.

US 6,140,496 relates to building blocks for preparing oligonucleotides carrying non-standard nucleobases that can pair with complementary non-standard nucleobases so as to fit the Watson-Crick geometry. The resulting base pair joins a monocyclic six membered ring pairing with a fused bicyclic heterocyclic ring system composed of a five member ring fused with a six member ring, with the orientation of the heterocycles with respect to each other and with respect to the backbone chain analogous to that found in DNA and RNA, but with a pattern of hydrogen bonds holding the base pair together different from that found in the AT and GC base pairs (a "non-standard base pair").

US 6,143,497 relates to a method for synthesizing diverse collections of random oligomers on particles by means of a general stochastic method. Also disclosed are identification tags located on the particles and used to facilitate identification of the sequence of the monomers in the oligomer.

US 6,165,717 relates to a general stochastic method for synthesizing random oligomers on particles. Also disclosed are identification tags located on the particles to facilitate identification of the sequence of the monomers in the oligomer.

US 6,175,001 relates to functionalized pyrimidine nucleosides and nucleotides and DNA's incorporating same. The modified pyrimidine nucleotides are derivatized at C5 to contain a functional group that mimics the property of a naturally occurring amino acid residues. DNA molecules containing the modified nucleotides are also provided. .

US 6,194,550 B1 relates to systematic polypeptide evolution by reverse translation, in particular a method for preparing polypeptide ligands of target molecules wherein candidate mixtures comprised of ribosome complexes or mRNA:polypeptide copolymers are partitioned relative to their affinity to the target and amplified to create a new candidate mixture enriched in ribosome complexes or mRNA:polypeptide copolymers with an affinity to the target.

US 6,207,446 B1 relates to methods and reagents for the selection of protein molecules that make use of RNA-protein fusions.

US 6,214,553 B1 relates to methods and reagents for the selection of protein molecules that make use of RNA-protein fusions.

WO 91/05058 relates to a method for the cell-free synthesis and isolation of novel genes and polypeptides. An expression unit is constructed onto which semi-random nucleotide sequences are attached. The semi-random nucleotide sequences are first transcribed to produce RNA, and then translated under conditions such that polysomes are produced. Polysomes which bind to a substance of interest are then isolated and disrupted; and the released mRNA is recovered. The mRNA is used to construct cDNA which is expressed to produce novel polypeptides.

WO 92/02536 relates to a method for preparing polypeptide ligands of target molecules wherein candidate mixtures comprised of ribosome complexes or mRNA:polypeptide copolymers are partitioned relative to their affinity to the target and amplified to create a new candidate mixture enriched in ribosome complexes or mRNA:polypeptide copolymers with an affinity to the target.

WO 93/03172 relates to a method for preparing polypeptide ligands of target molecules wherein candidate mixtures comprised of ribosome complexes or mRNA:polypeptide copolymers are partitioned relative to their affinity to the target and amplified to create a new candidate mixture enriched in ribosome complexes or mRNA:polypeptide copolymers with an affinity to the target.

WO 93/06121 relates to a general stochastic method for synthesizing random oligomers on particles. Also disclosed are identification tags located on the particles to facilitate identification of the sequence of the monomers in the oligomer.

WO 00/47775 relates to a method for generating RNA-protein fusions involving a high-salt post-translational step.

Additional references of relevance for present invention includes Bain et al. Nature, vol. 356, 1992, 537-539; Barbas et al. Chem. Int. Ed. vol. 37, 1998. 2872-2875; Benner Reviews; Blanco et al. Analytical Biochemistry vol. 163, 1987, 537-545; Brenner et al. Proc. Natl. Acad. Sci. Vol. 89, 1992, 5381-5383; Bresler et al. Biochimica et Biophysica Acta vol. 155, 1968, 465-475; Dewey et al. J. Am. Chem. Soc. Vol. 117, 1995, 8474-8475; Dietz et al. Photochemistry and photobiology vol. 49, 1989, 121-129; Gryaznov et al. J. Am. Chem. Soc. vol. 115, 1993, 3808-3809; Gryaznov et al. Nucleic Acids Research vol. 21, 1993, 1403-1408; Elmar Gocke Mutation Research vol. 248, 1991, 135-143; Haeuptle et al. Nucleic Acids Research, 14, 1986, 1427-1448; Hamburger et al. Biochimica et Biophysica Acta, 213, 1970, 115-123; Hamza A. El-Dorry Biochimica et Biophysica Acta vol. 867, 1986, 252-255; Herrera-Estrella et al. The EMBO Journal, 7, 1988, 4055-4062; Heywood et al. Biochemistry vol. 57, 1967, 1002-1009; Heywood et al. J. Biol. Chem. Vol. 7, 1968, 3289-3296; Hooper et al. Eur. J. Clin. Microbiol. Infect. Dis. Vol. 10, 1991, 223-231; Houdebine et al. Eur. J. Biochem., 63, 1976, 9-14; Johnson et al. Biochemistry vol.

25, 1986, 5518-5525; Kinoshita et al. Nucleic Acids Symposium Series vol. 34, 1995, 201-202; Leon et al. Biochemistry vol. 26, 1987, 7113-7121; Maclean et al. Proc. Natl. Acad. Sci. USA vol. 94, 1997, 2805-2810; Mattheakis et al. Proc. Natl. Acad. Sci. USA vol. 91, 1994, 9022-9026; Menninger et al. Antimicrobial Agents and Chemotherapy, 21, 1982, 811-818; Menninger. Biochimica et Biophysica Acta, 240, 1971, 237-243; Mirzabekov Methods in Enzymology vol. 170, 1989, 386-408; Nikolaev et al. Nucleic Acids Research vol. 16, 1988, 519-535; Noren et al. Science vol. 24, 1989, 182-188; Pashev et al. TIBS vol. 16, 1991, 323-326; Pargellis et al. The Journal of Biological Chemistry, 263, 1988, 7678-7685; Pansegrau et al. The journal of biological chemistry vol.265, 1990, 10637-10644; Peeters et al. FEBS Lett. vol. 36, 1973, 217-221; Roberts et al. Proc. Natl. Acad. Sci. USA vol. 94,1997, 12297-12302; Schmidt et al. Nucleic Acids Research vol. 25, 1997, 4797-4802; Schutz et al. Nucleic Acids Research, 4, 1977, 71-84; Solomon et al. Proc. Natl. Acad. Sci USA vol. 82, 1985,6470-6474; Sugino et al. Nucleic Acids Research, 8, 1980, 3865-3874; Tarasow et al. Nucleic Acids Sciences vol. 48, 1998, 29-37; Wiegand et al. Chemistry and Biology vol. 4, 1997, 675-683; and Wower et al. Proc. Natl. Acad. Sci. USA., 86, 1989, 5232-5236.

Summary of the Invention

The present invention solves in a general way the above-mentioned problems and short-comings of the prior art. The invention relates to a system for templating polymers in general, and the template enables templated synthesis of the polymers, allowing in preferred embodiments amplification of the polymer. The system therefore has the same overall characteristics as the natural system (information flow from template to templated molecule), as well as the characteristics of the recently invented ribosome-mediated systems (e.g., phage display), namely the physical link between template and templated molecule. However, the present invention does not involve ribosomes or tRNAs, and therefore allows templating of a wide array of different polymers, including polymers that cannot be synthesised in a natural system based on ribosome-mediated translation of nucleic acids.

The templating process of the invention has significant advantages over the prior art. As the amplification of the recovered molecules (i.e., their templates) can be done by a parallel process in which all the recovered templates are present in the

same compartment (e.g., reagent tube or microtiter-plate well), and where the molecules are proportionately amplified, no human intervention such as sequencing of the individual molecules is necessary. This is a huge advantage since a typical recovery after a first selection round involves e.g. 10^{10} different molecules, when the starting material is a library of e.g. 10^{15} molecules. When working with such high numbers of molecules, it is practically impossible to "amplify" 10^{10} molecules by copying the molecules one-at-a-time, i.e., to "amplify" the molecules in a serial process.

The present invention generally relates to templated molecules and complexes comprising such molecules linked to a template that has directed the template-directed synthesis of the templated molecule. In one aspect, the templated molecules and the complexes are obtainable according to the methods of the present invention.

The present invention also discloses methods for synthesizing such templated molecules and/or complexes, methods for targeting such molecules and/or complexes to a target species. The templated molecules are preferably synthesised from building blocks comprising a functional entity comprising a functional group and reactive group capable of covalently linking functional groups and forming a templated molecule. The functional entity of a building block is separated from a complementing element by a cleavable linker, or a selectively cleavable linker. The complementing element is capable of complementing a predetermined coding element of the template, thus ensuring a one-to-one relationship between a coding element - or a complementing element - and a functional entity, or a functional group.

Also disclosed are methods for identifying the sequence of functional groups of a templated molecule, as well as methods for therapy and diagnostic methods exploiting the templated molecules according to the invention.

The methods of the invention do not involve ribosome mediated translation of ribonucleic acids. Also, when the templated molecules are peptides comprising either i) exclusively α-amino acids, or ii) substantially exclusively naturally occurring amino acids, such as at least 80 percent, for example 90 percent, such as 95

percent, naturally occurring amino acids, the template does not comprise or essentially consist of a ribonucleic acid.

A template denotes a sequence of coding elements, wherein each coding element is linked to a neighbouring coding element. A complementing template denotes a sequence of complementing elements, wherein each complementing element is linked to neighbouring complementing element.

Following complementation of a coding element by a complementing element, or complementation of a plurality of coding elements by a plurality of complementing elements, each complementing element will define an appended functional group capable of being linked - without forming part of the complementing template itself - to a neighbouring functional group defined by a neighbouring complementing element. Accordingly, in one preferred embodiment, the functional group does not participate in the complementation of a coding element in so far as no direct reaction or hybridization takes place between the coding element and the functional group. The term "reaction" means any reactive contact that that results in the formation of an interaction - covalent or non-covalent - between the functional group and the coding element. In another embodiment, the functional group of a templated molecule forms part of the complementing template.

As each complementing element is capable of recognising a predetermined coding element of a template, and as each coding element in turn defines a predetermined functional group, the sequence of coding elements of the template will template the synthesis of the templated molecule comprising a predetermined sequence of covalently linked functional groups.

According to preferred embodiments of the present invention, it is possible

i) to link a templated molecule comprising a plurality of functional groups to the template that templated the synthesis of the templated molecule,

ii) to link neighbouring functional groups simultaneously with the complementation of neighbouring coding elements by complementing elements defining said functional groups,

iii) to link neighbouring functional groups after the complementation of neighbouring coding elements by complementing elements defining said functional groups,

iv) to link neighbouring functional groups simultaneously with the formation of a complementing template,

v) to link neighbouring functional groups after the formation of a complementing template,

vi) to cleave one or more links between complementing elements of a complementing template without cleaving links between functional groups of a templated molecule, and vice versa, and

vii) to cleave the at least one linker separating the at least one functional entity from the at least one complementing element of a building block without cleaving the complementing template,

viii) to cleave the at least one linker separating the at least one functional entity from the at least one complementing element of a building block without cleaving the link between the functional groups of the templated molecule, and

ix) to cleave the at least one linker separating the at least one functional entity from the at least one complementing element of a building block without cleaving the complementing template and without cleaving the link between the functional groups of the templated molecule.

Provided that complementation of neighbouring coding elements is achieved, the neighbouring, functional groups of the templated molecule are capable of being linked irrespective of whether a complementing template is formed. Also, it is possible to link neighbouring functional groups and subsequently cleave the cleavable linker separating the functional entity from the complementing element defining said functional entity without cleaving the link between neighbouring functional groups of a templated molecule. Cleavable linkers are cleavable under conditions wherein a selectively cleavable linker is not cleavable. Accordingly, it is

possible to cleave the cleavable linkers linking complementing elements and functional groups in a templated molecule without at the same time cleaving selectively cleavable linkers linking - in the same templated molecule - a subset of complementing elements and functional groups. It is thus possible to obtain a complex comprising a templated molecule and the template that has directed the template-mediated synthesis of the templated molecule, wherein the template and the templated molecule are linked by one or more, preferably one, selectively cleavable linker(s).

The generation of additional templated molecules can be directed by the template without any need for sequencing or any other form of characterisation. This is not possible using prior art "tags" generated by step-by-step synthesis. Accordingly, the complexes of the invention comprising a templated molecule linked to a template makes it possible to rapidly select and amplify desirable, templated molecules.

In a first aspect, the present invention provides a method for synthesising a templated molecule comprising a plurality of functional groups, said method comprising the steps of

i) providing at least one template comprising a sequence of n coding elements,

wherein each coding element comprises at least one recognition group capable of recognising a predetermined complementing element, and

wherein n is an integer of more than 1,

ii) providing a plurality of building blocks, wherein each building block comprises

a) at least one complementing element comprising at least one recognition group capable of recognising a predetermined coding element,

b) at least one functional entity comprising at least one functional group and at least one reactive group, and

c) at least one linker separating the at least one functional entity from the at least one complementing element,

iii) obtaining a complementing template by complementing a plurality of predetermined coding elements by contacting each of said coding elements with a complementing element capable of recognising said coding element, and

iv) obtaining a templated molecule comprising a sequence of covalently linked, functional groups by linking, by means of a reaction involving reactive groups, a functional group of at least one functional entity to a functional group of a neighbouring, functional entity,

wherein the templated molecule is capable of being linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, and

wherein the synthesis of the templated molecule does not involve ribosome mediated translation of a nucleic acid.

In another aspect, the present invention relates to a templated molecule, a plurality of the same or different templated molecules, wherein preferably each of the templated molecules are obtainable by a method for synthesizing templated molecules according to the present invention.

As the templated molecule and the template are separate entities capable of being linked by a single linker, the invention also relates to complexes comprising a templated molecule liked to the template that templated the synthesis of the templated molecule. The template capable of templating the synthesis of the templated molecule comprises either a sequence of coding elements, or a sequence of complementing elements, in which case the template is a complementing

template. Accordingly, it is possible to cleave links between functional groups of a templated molecule without cleaving a complementing template or template that templated the synthesis of the templated molecule.

In another aspect there is provided a method for synthesising a complex comprising a templated molecule linked to the template that templated the synthesis of the templated molecule, wherein the templated molecule and the complex comprising the templated molecule linked to the template that templated the synthesis of the templated molecule are obtainable by the method for synthesis thereof according to the invention.

In further aspects of the invention there is provided a composition comprising a plurality of templated molecules, wherein each or at least some of the templated molecules are linked to the template that templated the synthesis of the templated molecule, in which case there is provided a plurality of complexes each comprising a templated molecule linked to the template that templated the synthesis of the templated molecule. The compositions may also comprise a templated molecule and - unlinked thereto - the template that templated the synthesis of the templated molecule.

The amplifiability of the templated molecules of a library provides a library with a unique feature. This unique feature involves e.g. that a huge number of templated molecules can be screened by taking the library through repetitive processes of selection-and-amplification, in a parallel process where the library of molecules is treated as a whole, and where it is not necessary to characterise individual molecules (or even the population of molecules) between selection-and-amplification rounds.

It is possible according to various preferred embodiments of the invention to screen e.g. more than or about 10^3 different templated molecules, such as more than or about 10^4 different templated molecules, for example more than or about 10^5 different templated molecules, such as more than or about 10^6 different templated molecules, for example more than or about 10^7 different templated molecules, such as more than or about 10^8 different templated molecules, for example more than or about 10^9 different templated molecules, such as more than or about 10^{10} different

templated molecules, for example more than or about 10^{11} different templated molecules, such as more than or about 10^{12} different templated molecules, for example more than or about 10^{13} different templated molecules, such as more than or about 10^{14} different templated molecules, for example more than or about 10^{15} different templated molecules, such as more than or about 10^{16} different templated molecules, for example more than or about 10^{17} different templated molecules, such as more than or about 10^{18} different templated molecules.

As one may perform many repetitive rounds of parallel selection and parallel amplification processes, it is possible to enrich only e.g. 100 fold in each round, and still get a very efficient enrichment, of e.g. 10^{14} fold over a number of selection-and-amplification rounds (theoretically a 10^{14} fold enrichment is obtained after seven rounds each enriching 100 fold). To obtain a similar enrichment of 10^{14} fold using a non-amplifiable library, would require screening conditions allowing 10^{14} fold enrichment in one "round" - and this is not practically possible using state-of-the-art screening technologies. The templated molecules and/or the templates can furthermore be bound to a solid or semi-solid support.

In even further aspects the methods of the invention - individually or as a combination - relates to

a method for screening a composition of complexes or templated molecules potentially having a predetermined activity,

a method for assaying the predetermined activity potentially associated with the complexes or the templated molecules,

a method for selecting complexes or templated molecules having a predetermined activity,

a method for amplifying the template that templated the synthesis of the templated molecule having, or potentially having a predetermined activity, and

a method for amplifying the template that templated the synthesis of the templated molecule having, or potentially having, a predetermined activity, said method com-

prising the further step of obtaining the templated molecule in an at least two-fold increased amount.

In yet another aspect there is provided a method for altering the sequence of a templated molecule, including generating a templated molecule comprising a novel or altered sequence of functional groups, wherein the method comprises the step of mutating the template that templated the synthesis of the original templated molecule. The method preferably comprises the steps of

i) providing a first template capable of templating the first templated molecule, or a plurality of such templates capable of templating a plurality of first templated molecules,

ii) modifying the sequence of the first template, or the plurality or first templates, and generating a second template, or a plurality of second templates,

wherein said second template(s) is capable of templating the synthesis of a second templated molecule, or a plurality of second templated molecules,

wherein said second templated molecule(s) comprises a sequence of covalently linked, functional groups that is not identical to the sequence of functional groups of the first templated molecule(s), and optionally

iii) templating by means of said second template(s) a second templated molecule, or a plurality of such second templated molecules.

The above-mentioned method exploits that a templated synthesis (Figure 1) in one embodiment involves a single-stranded, modifiable intermediate in the form of a template. In the case where this template comprises a nucleotide strand comprising deoxyribonucleotides or ribonucleotides, most molecular biological methods can be applied to modify the template, and therefore to modify the templated polymer.

The below-mentioned list of molecular biological methods that can be applied to the templated polymers of this invention is therefore far from comprehensive, but merely

serves to illustrate that almost any relevant molecular biological method can be applied to the templated polymers as a result of the present invention.

In cases where nucleotides with non-natural bases are part of the template, some of the molecular biology methodologies may not be applicable. This will primarily depend on the substrate specificty of the enzymes involved (e.g., the Taq DNA polymerase in a PCR reaction; restriction enzyme in USE protocol; etc). Also, methods that involve an in vivo step (e.g., transformation of E. coli for amplification of plasmid DNA) may only have a limited feasibility for those nucleotides. Several nucleotides with non-natural bases are, however, known to be incorporated into oligonucleotides by several wildtype and mutant polymerases, and therefore, the use of nucleotides with non-natural bases does not seriously limit the number of in vitro molecular biology methods that can be applied to templated molecules.

Table 1. Molecular Biology applicable to the templated polymers of this invention

- In vivo and in vitro amplification, recombination and mutagenesis
- Kunkel site-directed mutagenesis, using one or multiple (e.g., 50) different mutagenic oligos at below-saturating concentrations, i.e., generating a combinatorial library
- USE (Unique Site-directed Elimination), using one or multiple (e.g., 50 different mutagenic oligos) at below-saturating concentrations, i.e., generating a combinatorial library
- PCR (Polymerase Chain Reaction)
- LCR (Ligase Chain Reaction)
- PCR shuffling, including family shuffling (shuffling sequences containing blocks with particular homology), and directed shuffling where oligos are spiked into the reaction to direct the shuffling process in a certain direction
- Other types of shuffling, e.g. homologous recombination in yeast; shuffling protocols as developed at the companies Phylos, Energy Biosystems, Diversa and by Frances Arnold.
- Cassette mutagenesis
- Other polymerase- or PCR-based methods, e.g., overlap extension, gene synthesis, and error-prone PCR
- Chemical or UV-induced mutagenesis
- Wildtype or variant template synthesis and translation into templated polymer (wildtype in this respect means the template sequence that will template the synthesis of the known ("wildtype") polymer; variant in this respect means a partly randomised or spiked template sequence that will template the synthesis of a variant of the known polymer)
- Specific cleavage by restriction enzymes
- Ligation by DNA or RNA ligases; "gene splicing"
- Affinity selections (using the template-templated polymer complex)
- Sequencing
- Arraying the polymers on "DNA chips", by using the template as a tag that binds a DNA array

Instead of isolating the (underivatized) template strand, it may be desirable to apply the molecular biological methods to either the template-complementing template double-helix or to the derivatized complementing template. The derivatized template may at this point contain unpolymerized functional entities; polymerized functional entities; or a trace left behind from the cleaving of the linker that connected the functional entity and the complementing element. Many polymerases

and other enzymes are known to accept DNA- or RNA-templates with a high degree of derivatization. Therefore, many in vitro methods involving polymerases and other enzymes are likely to be feasible using the (derivatized) complementing template as starting point. It will primarily depend on the substrate- or template specificity of the enzymes involved whether it will be feasible to use the derivatized complementing template as a starting point for the molecular biological method in question. The skilled person will be capable of evaluating the feasibility of various practical approaches in this respect.

The present invention also pertains to building blocks used for synthesising the templated molecule and to complexes comprising such building blocks. In another aspect there is provided the use of a building block for the synthesis of a templated molecule according to the invention. In a preferred embodiment of this aspect, the templated molecule comprises or essentially consists of a molecular entity capable of binding to another molecular entity in the form of a target moleculer entity or a binding partner.

The templated molecule is preferably a medicament capable of being administered in a pharmaceutically effective amount in a pharmaceutical composition to an individual and treating a clinical condition in said individual in need of such treatment.

In other aspects of the invention there are provided a pesticidal composition, an insecticidal composition, a bacteriocidal composition, and a fungicidal composition, as well as methods for preparing such compositions and uses thereof, wherein each of said compositions comprise a templated molecule according to the invention in an amount effective to achieve a desired effect.

In still further aspects there is provided a method for identifying a pharmaceutical agent, or a diagnostic agent, wherein said method comprises the step of screening a plurality of drug targets with at least one predetermined, templated molecule, and identifying a pharmaceutical agent, or a diagnostic agent, in the form of candidate templated molecules capable of interacting with said drug targets.

In yet another aspect there is provided a method for identifying a target, including a drug target, wherein said method comprises the step of screening a plurality of lig-

ands or receptor moieties with at least one predetermined, templated molecule, and identifying drug targets in the form of ligands or receptor moieties capable of interacting with said templated molecules.

The present invention also relates to any isolated or purified templated molecule having an affinity for a predetermined target, including a drug target, as well as to targets, including drug targets, in the form of ligands, receptor moieties, enzymes, cell surfaces, solid or semi-solid surfaces, as well as any other physical or molecular entity or surface having an affinity for a predetermined templated molecule.

In even further aspects of the invention there is provided a method for treatment of an individual in need thereof, said method comprises the step of administering to the individual a pharmaceutically effective amount of a molecule identified by a method of the present invention and having an affinity for a predetermined target, including a drug target.

In a still further aspect there is provided a method for treatment of an individual in need thereof, said method comprises the step of administering to the individual a pharmaceutically effective amount of an isolated or purified ligand or receptor moiety having an affinity for a predetermined templated molecule according to the invention. The isolated or purified ligand or receptor moiety is preferably identified by the above-mentioned method of identification of the invention.

Brief Description of the Figures

The following symbols are used in the following figures to indicate general characteristics of the system: In figures 1, 7C, 8C, 11, 11 ex.1, 12, 13, 14, 14 ex. 1 – 2, 15, 15 ex. 1 – 7, 16, 16 ex. 1, 17, 17 ex. 1 – 2, 18, 18 ex. 1 – 3, 19, 20, and 21A, a long horizontal line symbolizes a template, complementing template or the complex of the template with the complementing template. For clarity, in some of the figures only the polymerization step, not the activation step, has been included. Rx denotes functional groups.

Figure 1. Chemical Display of Templated Molecules - The principle.

The protocol for the chemical display of templated molecules can be divided into 6 steps, i) incorporation, ii) polymerization, iii) activation, iv) selection/screening, v) amplification, and vi) characterization. Incorporation involves the incorporation of building blocks into the complementing template, which sequence is determined by the template.

Incorporation may be mediated by enzymes such as polymerase or ligase. The template comprises primer binding sites at one or both ends (allowing the amplification of the template). The remaining portion of the template may be of random, partly random or predetermined sequence. The complementing elements preferably comprises of a functional entity, a complementing element and a linker connecting the functional entity and the complementing element. Detailed examples of selected complementing elements, their incorporation, polymerization and activation are shown in (Figure 7 and 8).

Polymerization involves reactions between the incorporated building blocks, thereby forming covalent bonds between the functional entities, in addition to the functional bonds that already exist between the complementing elements.

Activation involves cleaving some, all but one, or all of the linkers that connect the sequence of functional entities to the template or complementing template having templated the templated molecule comprising the functional entities. Activation may also involve separating the template and the complementing template without cleaving the linkers connecting the functional entities and the complementing template.

Selection or screening involves enriching the population of template-templated molecule pairs for a desired property.

Amplification involves producing more of the template-templated molecule pairs, by amplification of the template or complementing template, and producing more of the template-templated molecule pairs, for further rounds of selection/screening, or for sequencing or other characterization.

Cloning and sequencing involves the cloning of the isolated templates or complementing templates, followed by characterization. In some cases, it may be desirable to sequence the population of isolated templates or complementing templates, wherefore cloning of individual sequences are not required.

Figure 2A and 2B. An expanded set of base pairs.

The figure discloses a set of natural and non-natural base pairs that obeys Watson-Crick hydrogen-bonding rules. The base pairs are disclosed in US 6,037,120, incorporated herein by reference.

Figure 3. A monomer building block.
A building block comprises or essentially consists of a functional entity, connected through a selectively cleavable linker to a complementing element. Each complementing element has two reactive groups (type I), which may react with two other complementing elements. The complementing element contains a recognition group that interacts with a complementary coding element (coding element not shown). The functional entity in this example comprises or essentially consists of two reactive groups (type II), which may react with reactive groups of other functional entitie(s), and a functional group, also called a functionality. The reactive groups of type II, and the molecular moiety that connects them, will become (part of) the backbone in the resulting encoded polymer.

Figure 4. A monomer building block with only one reactive group type II.
A building block comprises or essentially consists of a functional entity, connected through a selectively cleavable linker to a complementing element. Each complementing element has two reactive groups (type I), which may react with other complementing elements. The complementing element contains a recognition group that interacts with a complementary coding element (coding element not shown). The functional entity in this example comprises or essentially consists of a reactive group type II, which may react with reactive groups of other functional entities, and a functional group, also called a functionality. The reactive group type II will become (part of) the backbone in the resulting encoded polymer.

Figure 5. Building blocks and the polymers resulting from template directed incorporation of the building blocks and their polymerization and activation
Figure 3 discloses a detailed description of features of individual building blocks. Three different complementing elements are shown, each linked to a specific functional entity. The right half of the figure includes the template which directs the incorporation of the building blocks by complementary base pairing.
A). The reactive groups type I of the complementing element react, whereby a part of the reactive group is lost (e.g., PP_i in the incorporation of nucleoside triphos-

phates). In the shown example, the polymerization of reactive groups type II also results in loss of part of the reactive groups. The backbone of the resulting polymer comprises or essentially consists of part of the original reactive groups type II and the molecular entity that connects the reactive groups. Part of the linker remains attached to the functional entity.

B). The reactive groups type I react as in (A). The reactive groups type II do not react directly, but rather a "bridging molecule" is added. Upon reaction with this bridging molecule, part of the reactive group is lost. The cleavable linker used in this example is a so-called "traceless linker" and therefore the functional entity is released with no trace of the linker molecule.

C). Incorporation in this case does not involve coupling of the individual complementing elements, i.e., does not lead to the reaction of the reactive groups type I. The reactive groups type II react with bridging molecules as in (B).

D). The functional entity contains only one reactive group type II. The reactive group type II reacts with a bridging molecule.

Figure 6. A derivatized nucleotide as building block

The building block comprises or essentially consists of the complementing element (the nucleotide) and a functional entity (in this case a dicarboxylic acid) connected by means of a selectively cleavable linker (here a disulfide). The reactive groups type I of the nucleotide are the triphosphate and the hydroxyl group, as indicated. The recognition group of the nucleotide is the base. The functional entity comprises or essentially consists of a functional group (a hydroxyl), two reactive groups type II (carboxylic acids), and a backbone structure (aromatic ring) connecting the two reactive groups. Finally the linker (disulfide) is cleavable by for example DTT.

Figure 7. C-terminal tagging of a β-dipeptide - incorporation, polymerization and activation.

A) Structures of the primer and two monomer building blocks. The initiator molecule is attached to the 5-position of the 3'-terminal dU of the primer. The initiator is a Fmoc-protected amine. The dUTP-derivative carries a photoprotected hydroxyl group. The hydroxyl group is coupled to the N-thiocarboxyanhydride (NTA) ring structure. The dATP-derivative is modified at the 7 position. A photoprotected amine is coupled the NTA.

B) The primer (which is annealed to the template, not shown in figure) is extended from its 3'-end through incorporation of the dUTP and dATP by a polymerase. Then the initiator is activated by piperidine, which releases the primary amine. The primary amine attacks the neighboring NTA, which opens the NTA rings structure, releases CSO, and as a result, produces a primary amine. This primary amine now attacks the next NTA unit in the array. As a result, a polymer, attached through its functional groups (OH and NH_2) to the DNA strand, is formed. Finally, the linkers connecting the DNA strand with the NTA units, are cleaved. The resulting polymer in this case is a β-peptide, carrying the functional groups OH and NH_2, encoded by the DNA sequence dUdA. In the shown example, the sequence 5'-dUdA-3' encodes a β-peptide in the C-terminal to N-terminal direction, as opposed to Natures encoding system where 5' to 3' RNA encodes an α-peptide in the N- to C-terminal direction. The β-peptide is attached to the encoding DNA through its C-terminal end.

C) Schematic representation of the incorporation, polymerisation and activation. The encoded polymer becomes attached to the encoding molecule (DNA) through the initiator molecule.

Figure 8. N-teminal tagging of a β-dipeptide - incorporation, polymerization and activation.

A) Structures of the primer, two monomer building blocks, and an oligo. The initiator molecule is attached to the 5-position of the 3'-terminal U of the primer. The primer is complementary to the upstream part of the template. The initiator is a Fmoc-protected amine. The UTP-derivative carries a photo-protected hydroxyl group. The hydroxyl group is attached to the N-thiocarboxyanhydride (NTA) ring structure. The ATP-derivative is modified at the 7 position. A photo-protected amine is attached to the NTA. The oligo is complementary to the downstream sequence of the template. The oligo carries a reactive thioester attached to the U at the oligo's 5'end. The stability of the thioester in water can be modified as desired by changing the structure of the thioester-component (in the example, the thiol-component is a thiophenol).

B) The primer (which is annealed to the template, not shown in figure) is extended from its 3'-end through incorporation of the UTP and ATP by a polymerase. Then the initiator is activated by piperidine, which releases the primary amine. The primary amine attacks the neighboring NTA, which opens the NTA rings structure, releases CSO, and as a result, produces a primary amine. This primary amine now attacks the next NTA unit in the array. As a result, a polymer, attached through its functional groups (OH and NH_2) to the RNA strand, is formed. Finally, the linkers connecting the RNA strand with the NTA units are cleaved. The resulting polymer is a β-peptide, carrying the functional groups -OH and -NH_2, encoded by the ribonucleic acid sequence UA. The sequence 5'-UA-3' encodes a β-dipeptide in the N-terminal to C-terminal direction, similar to the way that Nature encodes α-peptides. The β-peptide is attached to the encoding RNA through its N-terminal end.

C) Schematic representation of the incorporation, polymerisation and activation. Upon cleavage of a subset of linkers, the encoded polymer becomes attached to the downstream oligonucleotide.

Figure 9. Nucleotide–derivatives that are known to be incorporated into RNA or DNA strands by DNA or RNA polymerases.

Top: Nucleotide, the four bases and the site of attachment of the molecular moiety (R).

Center: Nucleotides with appendices (R) that are accepted as substrates by polymerases.

Bottom: Nucleotides with appendices (R) that may be used with the present invention. Compound (a) would be used in for example fill-in experiments (see Figure 15). Compound (b) would be used for example in zipping polymerization reactions (see Figure 14 and 14, example 1). Compound (c) would be used for example in ring-opening polymerization reactions (see Figure 18 and 18, example 1).

Figure 10. Cleavable linkers and protection groups.

Cleavable linkers and protection groups, agents that may be used for their cleavage and the products of cleavage.

Figure 11. Polymerization by reaction between neighboring reactive groups type II.

For clarity, only the polymerization reaction (and not the activation) is shown in the figure. X represents the reactive groups type II of the functional entity. In this case the two reactive groups type II are identical.

Polymerization (reaction of X with X to form XX) either happens spontaneously when the monomer building block has been incorporated , or is induced by a change of conditions (e.g. pH), or by the addition of an inducing factor (chemical or UV exposure, for example)

Figure 11 ex.1. Coumarin-based polymerization.

Light-induced reaction of the coumarin units, followed by activation (cleavage of the linker), results in a polymer backbone of aromatic and aliphatic ring structures. Examples of functional groups (phosphate, carboxylic acid and aniline) are shown.

Figure 12. Polymerization between neighboring non-identical reactive groups type II.

In this example, X may react with Y but not another X. Likewise, Y does not react with Y. Polymerization can either happen during the incorporation of building blocks (as shown in the figure), or after incorporation of several building blocks.

Figure 13. Cluster formation in the absence of directional polymerisation.

When the incorporated monomers are not fixed with regard to rotation about the bond that links the functional entities to the complementing elements, cluster formation may result, as shown in the figure.

This represents a significant problem for longer polymers. The problem may be solved by (i) fixing the incorporated monomers in a preferred orientation which does not allow X and Y (reactive groups type II) to exchange positions in the array (e.g., by coupling the functional entity and the complementing element via a double bond or two bonds, e.g.. coupling the functional entity both to the base and the ribose of a nucleotide, or to the two bases of a dinucleotide), (ii) employing directional polymerisation ("zipping", see for example figure 17), or (iii) setting up conditions that ensure that the monomers react during or right after incorporation into the complementing template, i.e., each monomer reacts with the previously incorporated

monomer before the next monomer is incorporated (see for example Figure 14, with example).

Figure 14. Zipping-polymerization and simultaneous activation.
Polymerization results in activation of the polymer. The geometry of the reaction between X and Y is in this example the same for all monomers participating in the polymerization

Figure 14, example 1. Simultaneous incorporation, polymerisation and activation - formation of peptides.

(A). Nucleotide derivatives, to which amino acids thioesters have been appended, are incorporated. During or after incorporation of a nucleotide-derivative, the amine attacks the carbonyl of the (previously incorporated) neighboring nucleotide. This results in formation of an amide bond, which extends the peptide one unit. When the next monomer is incorporated, this may attack the thioester carbonyl, resulting in cleavage of the dipeptide from the nucleotide, to form a tripeptide. The process continues further downstream the complementing template, until incorporation of nucleotide derivatives stops. Importantly, the geometry of the nucleophilic attack remains unchanged. As the local concentration of nucleophilic amines is much higher on the template than in solution, reactions in solution is not expected to significantly affect the formation of the correct encoded polymer. Furthermore, the reactivity of the amine with the ester may be tuned in several ways. Parameters that will affect the reactivity include: (i) pH and temperature, (ii) length, point of attachment to the nucleotide, and characteristics (charge, rigidity, hydrophobicity, structure) of the linker that connects the ester and the nucleotide, (iii) nature of ester (thio-, phospho-, or hydroxy-ester); (iv) the nature of the substituent on the sulfur (see (B) below). In addition, the efficiency of correct polymer formation is also affected by the rate of incorporation and rate of reaction once incorporated. The rate of incorporation is determined by kcat and Km. The kcat and Km values may be tuned by changing the conditions (pH, concentration of nucleotides, salts, templates and enzymes), by choice of enzyme, or by changing the characteristics of the enzyme by protein engineering . Also, the nature and size of the nucleotide-derivatives may influence its rate of incorporation.

This general scheme involving incorporation, polymerisation and activation during or right after building block incorporation, can be applied to most nucleophilic polymerisation reactions, including formation of various types of peptides, amides, and amide-like polymers (e.g., mono-,di-, tri-, and tetra-substituted α-, β-, γ-, and Ω-peptides, polyesters, polycarbonate, polycarbamate, polyurea), using similar structures.

(B). Four different thioesters with different substituents and therefore different reactivity towards nucleophiles.

Figure 14, example 2. Simultaneous incorporation, polymerization and activation - formation of a polyamine.

This figure shows a "rolling-circle polymerization reaction" where the chain containing the nucleophilic center attacks the electrophile attached to the DNA-part, using this DNA-part as the leaving group.

Figure 15. "Fill-in" polymerization (symmetric XX monomers).

Fill-in polymerization by reaction between reactive groups type II ("X" in the figure) and bridging molecules (Y-Y) in figure).

For clarity, only the polymerization reaction (not the activation) is shown in the figure. The thick line represents double or single stranded nucleic acid or nucleic acid analog. X represents the reactive groups type II of the functional entity. In this case the two reactive groups type II are identical. (Y-Y) is added to the mixture before, during or after incorporation of the monomer building blocks. Likewise, significant reaction between X and Y may take place during or after incorporation of the monomers.

Figure 15, ex.1. Poly-imine formation by fill-in polymerization.

Dialdehyde is added in excess to incorporated diamines. As a result, a poly-imine is formed. In the example, the polymer carries the following sequence of functional groups: cyclopentadienyl, hydroxyl, and carboxylic acid.

Figure 15, example 2. Polyamide formation.

After incorporation of nucleotides to which have been appended di-amines, EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide) and dicarboxylic acid is added in excess to the primary amines on the oligonucleotide using standard coupling condi-

tions. Alternatively, a di-(N-hydroxy-succinimide ester) may be added in excess, at a pH of 7-10. As a result, two amide-bonds are formed between two neighboring nucleotide-appendices. After this polymerisation, the appendices are separated from the oligonucleotide backbone (activation), leaving one linker intact, and the protected functional groups are deprotected to expose the functional groups. The final result is a DNA-tagged polyamide.

An alternative route to polyamides would be to incorporate nucleotides to which had been appended di-carboxylic acids, and then add di-amines and EDC, to form amide bonds between individual nucleotides of the oligonucleotide. Alternatively, the nucleotide derivatives might contain N-hydroxy-succinimidyl (NHS) esters, which would react with the added amines without the need to add EDC. However, this latter method may be problematic in the case where incorporation is mediated by a polymerase, as the NHS-esters probably would react with amines on the polymerase, potentially inhibiting the activity of the polymerase.

(A). The backbone of the resulting polymer comprises or essentially consists of amide-bonded aromatic rings. The substituents of this example are a protected primary amine, a branched pentyl group, a tertiary amine and a pyrimidyl. The primary amine is protected in order to avoid its reaction with the dicarboxylic acid. Appropriate protecting groups would be for example Boc-, Fmoc, benzyloxycarbonyl (Z, cbz), trifluoracetyl, phthaloyl, or other amino protecting groups described e.g. in (T. W. Green and Peter G. M. Wuts (1991), Protective Groups in Organic Synthesis).

(B). The backbone comprises or essentially consists of aromatic rings, connected by amide bonds. The substituents are indanyl, diphenylphosphinyl, carboxamidoethyl and guanidylpropyl, the latter two representing the asparagine side chain, and the arginine side chain, respectively. The guanidyl function is protected, as it is more reactive than standard amines. An appropriate protecting group would be Mtr (4-methoxy-2,3,6-trimethylbenzenesulfonyl), Mts (mesitylene-2-sulfonyl) or Pbf (2,2,4,6,7-pentamethyldihydro-benzifuran-5-sulfonyl).

Figure 15, example 3. Polyurea formation.

The incorporated nucleotide derivatives react with phosgen or a phosgen-equivalent such as CDI to form a polyurea. The linkers are cleaved and the protected hydroxyl is deprotected.

Appropriate leaving groups (Lv) are chloride, imidazole, nitrotriazole, or other good leaving groups commonly employed in organic synthesis

Figure 15, example 4. Chiral and achiral polyurea backbone formation.
In this example, the functional group Rx is used as a cleavable linker, that generates the desired functional group upon activation. In both (A) and (B), a polyurea is formed.
In (A), the functional group is attached to the backbone via a chiral carbon. The hydrogen on this carbon is drawn to emphasize this. Before polymerisation, there is free rotation about the bond connecting the chiral carbon and the functional group. When the reactive groups type II (the amines) react with the phosgen equivalent (e.g., a carbonyldiimidazole) to form the polymer, the building blocks may be inserted in either of two orientations (as indicated by the position of the hydrogen, left or right). As a result, each residue of the polymer has two possible chiral forms. Therefore, a given encoding molecule will encode a polymer with a specific sequence of residues, but an encoded polymer of 5 or 15 residues will have $2^5 = 32$ or $2^{15} = 32768$ stereoisomers, respectively. In certain cases it may be advantageous to incorporate such additional structural diversity in the library (for example when the polymer is relatively short). In other cases such additional diversity is not desirable, as the screening efficiency may become compromised, or it may become too difficult to deconvolute the structure of a polymer that has been isolated in a screening process, together with the other stereoisomers encoded by the same encoding molecule (for example when the polymer is long).
In (B), the chiral carbon of (A) has been replaced by a nitrogen. As a result, the resulting polymer backbone is achiral, and the encoding molecule encodes one specific structure.

Figure 15, example 5. Polyphosphodiester formation.
The incorporated nucleotide derivatives react with the activated phosphodiester to form a polyphosphodiester. Then the linkers are cleaved, resulting in a polyphosphodiester, attached through a linker to the encoding molecule.
An example of an appropriate leaving groups (Lv) is imidazole.

Figure 15, example 6. Polyphosphodiester formation with one reactive group type II in each monomer building block.
Each incorporated nucleotide contains an activated phosphodiester. Upon addition of a dihydroxylated compound such as 1,3-dihydroxypyridine, a functionalised poly-

phosphodiester is formed. Finally, the functional groups Rx are liberated from the complementing template by cleavage of the protection groups/cleavable linker that connected them to the oligonucleotide.

Figure 15, example 7. Pericyclic, "fill-in" polymerization.
After incorporation of the nucleotide-derivatives, 1,4-benzoquinone is added in excess, resulting in the formation of a polycyclic compound. Finally, the polymeric structure is activated by cleaving the linkers that connect the polymer to the nucleotides, except for one (non-cleavable) linker which is left intact.

Figure 16. "Fill-in" polymerization (asymmetric XS monomers).
Fill-in polymerization by reaction between reactive groups type II ("X" and "S" in the figure) and bridging molecules (T-Y) in figure).
For clarity, only the polymerization reaction (not the activation) is shown. The thick line represents double or single stranded nucleic acid or nucleic acid analog. X and S represent the reactive groups type II of the functional entity. In this case the two reactive groups type II are non-identical. (T-Y) is added to the mixture before, during or after incorporation of the monomer building blocks. Likewise, significant reaction between X and Y, and between S and T may take place during or after incorporation of the monomers.

Figure 16, example 1. Fill-in polymerization by modified Staudinger ligation and ketone-hydrazide reaction.
The reactive groups (type II) X and S of the building blocks are azide and hydrazide. The added molecule that fills the gaps between the building blocks carry a ketone and a phosphine moiety. The reactions between a ketone and a hydrazide, and between a azide and a phosphine, are very chemoselective. Therefore, most functional groups Rx can be employed without the need for protection during the polymerization reactions. Examples for the molecular moieties R, R1, X and Y may be found in (Mahal et al. (1997), Science 276, pp. 1125-1128; Saxon et al. (2000), Organic Letters 2, pp. 2141-2143).

Figure 17. "Zipping" polymerization.
The initiator molecule (typically located at one of the ends of the nascent polymer) is activated, for example by deprotection or by a change in pH. The initiator then re-

acts with the reactive group X of the neighbouring monomer. This activates the reactive group Y for attack on the neighbouring X. Polymerisation then travels to the other end of the molecule in a "zipping" fashion, until all the desired monomers have been connected. The activation of the initiator (and reactive groups Y) may be both for attack by it on the neighbouring reactive group, or activation of it for attack by the neighbouring reactive group.

Figure 17, example 1. Radical polymerisation.
The initiator molecule, an iodide, is activated by the addition of a radical initiator, for example ammonium persulfate, AIBN (azobis-isobutyronitrile) or other radical chain reaction initiators. The radical attacks the neighboring monomer, to form a new radical and a bond between the first two monomers. Eventually the whole polymer is formed, and the polymer may be activated, which simultaneously creates the functional groups Rx.

Figure 17, example 2. Cationic polymerisation.
A cation is created by the exposure of the array to strong Lewis acid. The double bond of the neighbouring monomer reacts with this cation, whereby the positive charge migrates to the neighbouring monomer. Eventually the whole polymer is formed, and finally it is activated.

Figure 18. Zipping polymerization by ring opening.
The initiator reacts with the reactive group X in the ring structure, which opens the ring, whereby the reactive group Y in the same functional entity is activated for reaction with a reactive group X in a neighboring functional entity.

Figure 18, example 1. "Zipping" polymerization of N-thiocarboxyanhydrides, to form β-peptides.
After incorporation of the building blocks, the initiator is deprotected. The primary amine then attacks the carbonyl of the neighbouring N-thiocarboxyanhydride (NTA) unit. As a result, CSO is released, and a primary amine is generated. This amine will now react with the next NTA unit in the array, and eventually all the NTA units will have reacted, to form a b-peptide. Finally, the oligomer is activated.
A number of changes to this set-up can be envisaged. For example, instead of thiocarboxyanhydrides, one might use carboboxyanhydrides. The initiator might be

protected with a base- or photolabile group. If a base-labile protection group is chosen, the stability of the carboxyanhydride must be considered. At higher pH it may be advantageous to use carboxyanhydrides rather than thiocarboxyanhydrides. Finally, the initiator might be unprotected and for example coupled to the primer. In this case the concentration of the initiator in solution will be very low (typically nanomolar to micromolar), wherefore only an insignificant amount of initiator will react with the carboxyanhydrides. After or during incorporation of the building blocks the local concentration of initiator and carboxyanhydride will be much higher, leading to efficient polymerization.

Other types of peptides and peptide-like polymers (e.g., mono-,di-, tri-, and tetra-substituted α-, β-, γ-, and Ω-peptides, polyesters, polycarbonate, polycarbarmate, polyurea) can be made, using similar cyclic structures. For example, α-peptides can be made by polymerization of 5-membered carboxyanhydride rings.

Figure 18, example 2. "Zipping" polymerization of 2,2-diphenylthiazinanone units to form β-peptides.

The deprotected nucleophile, a primary amine, attacks the carbonyl of the neighboring thioester, thereby forming an amide bond. The released thiol reorganizes, to form a thioketone. As a result a free primary amine is generated, which attacks the carbonyl of a neighboring thioester, etc. Eventually an α-substituted β-peptide is formed, linked through its C-terminal end. The reactivity of the primary amine with the ester may be modified for example by the choice of ester (thioester or regular ester), pH during the polymerization reaction and the choice of substituents on the aromatic ring(s). The relative reactivity of the secondary amine contained in the cyclic moiety and the primary amine released upon ring-opening, may be adjusted by the bulk at the carbon between the secondary amine and the thioester. For example, replacing the two aromatic rings with one aromatic ring will decrese the bulk around the secondary amine, making it more nucleophilic, whereas the nucleophilicity of the primary amine that is formed upon ring-opening is not affected by the bulk at this position. Other peptides and amide-like polymers may be formed by this principle. For example, γ-peptides may be formed by polymerization of 7-membered thiazinanone rings.

Figure 18, example 3. Polyether formation by ring-opening polymerisation. The initiator is deprotected by for example base or acid. The formed anion the nattacks the epoxide of the neighboring monomer, to form a ether-bond. As a result, an anion is formed in the neighboring unit. This attacks the next monomer in the array, and eventually the full-length polyether has been formed. Depending on the conditions the attack will be at the most or least hindered carbon of the epoxide (under acidic or basic conditions, respectively).
In the final step, the encoded polyether is activated. In this case, the polymer is fully released from the encoding molecule. The screening for relevant characteristics (e.g., effect in a cell-based assay or enzymatic activity) may be performed in microtiter wells or micelles, each compartment containing a specific template molecule and the templated polyether, in many copies. In this way, the template and templated molecule is physically associated (by the boundaries of the compartment), and therefore the templates encoding polyethers with interesting characteristics may be collected from those compartments, pooled, amplified and "translated" into more copies of polyethers which may then be exposed to a new round of screening.

Figure 19. Zipping-polymerization and activation by rearrangement.
The initiator is activated for attack by Y. Reaction of initiator and Y results in release of the initiator from the complementing element. Upon reaction with the initiator, a rearrangement of the building block molecule takes place, resulting in activation of X for reaction with Y. After a number of reactions and rearrangements, a polymer has been formed.

Figure 20. Zipping-polymerization and activation by ring opening.
Reaction of the initiator with X in the ring structure opens the ring, resulting in activation of Y. Y can now react with X in a neighboring functional entity. As a result of ring-opening, the functional entities are released from the complementing elements.

Figure 21. Directional polymer formation using fixed functional units.

(A) The functional entity of a building block may be attached to the complementing element through two linkers. This may fix the functional entity in a given orientation relative to the complementing template. As a result, rotation around the linker that connects functional entity and complementing

element (as depicted in figure 13) is not possible, and cluster formation therefore unlikely.

(B) Two linkers connect the two bases of a dinucleotide-derivative with the functional unit, which in this case is a dipeptide. Incorporation of such dinucleotide derivatives into a double helical structure will position the amine (X in (A) above) in proximity to the ester (Y in (A) above). This ester may be activated, for example as a N-hydroxysuccinimide ester. After reaction of the amine and the ester, a polypeptide is formed. This polypeptide will be a directional polymer, with N-to-C-terminal directionality. In the present case, the polymerisation reaction will cleave the ester from the base to which it is linked. Therefore, activation of the formed polymer only requires cleavage of the linker that connects the base at the 3'-end of the dinucleotide with the amino-terminal end of the functional entity.

Rotational fixation of the functional entity relative to the complementing element may be achieved in other ways. For example, the functional entity may be coupled to the complementing element through a double bond, or it may be attached through two bonds to the base and ribose moity of a nucleotide, respectively, or it may be coupled to different positions on the ribose or base. Finally, it is also possible to link to the phosphate moity, especially of a dinucleotide.

Figure 22. Templated polymers.

A non-exhaustive and non-limiting list of oligomers that may be templated by the various principles described in the present invention. The list refers to any linear, branched or cyclic structure that contains one or more of the backbone structures listed, and/or contain several bonds of the same kind (e.g. amide bonds). Heteropolymers (hybrids of different polymer types) can also be templated by the present invention.

Figure 23. Reactive groups of type II (polymer precursors).

A list of some of the precursors (reactive groups type II) that may be used in the templated synthesis of various polymers.

Figure 24. Functional groups.

A list of some of the functional groups, R_x (functionalities), that may be used with the templating schemes in the present invention. The functional groups may have to be protected during incorporation, polymerization, and/or activation, or may have to be introduced post activation.

Figure 25. Polymers and the functional entities required to make them. The table provides examples of polymers that may be templated according to the principles described in the present invention. For each polymer, a suggested set of reactive groups type II (of the functional entity), a linking molecule or catalyst for the polymerization reaction where appropriate, and a reference to relevant figures, are provided.

Figure 26. Templating of molecules - principle and variations.

In the figures 26-27, 29-31, 33-35, 37-49, and 53, the template, the complementing template, both the template and the complementing template, or a complementing element is indicated by a horizontal (bold) line. In figures 26-28, 35-37, and 39, a circle is used to indicate a functional entity.

A. Monomer building blocks used in this figure. A black dot indicates a cleavable linker.
B. General principle.
Step 1 - Incorporation. The monomer building blocks are specifically incorporated into a complimentary template, by specific interaction between coding elements (of the template) and complementing elements (of the monomer building blocks).
Step 2 – Reaction. A reaction is induced by which functional entities (FE) of the individual monomer building blocks become coupled, by reaction of reactive groups type II.
Step 3 – Activation. Some or all of the linkers connecting the FE units with complementing elements are cleaved, thereby partly or fully releasing the templated molecule.
Step 4 (not shown in figure) – Screening, Amplification and Modification. The template-templated molecule complexes may be taken through a screening process that enriches the pool for complexes with desired fea-

tures. Then the templates of the enriched pool may be amplified and modified, by e.g. mutagenic PCR, and the templated molecules regenerated by performing step 1-3.

C. Templating of linear, branched and circular templates.
Linear, branched and circular templates may generate linear, branched and circular templated molecules. In the example shown, the branched template may be generated by incorporation of a modified nucleotide (e.g., carrying a thiol) into an oligonucleotide, followed by reaction with an oligonucleotide containing a thiol-reactive component (e.g., a maleimide-unit at one end). The circular template may likewise be a oligonucleotide, carrying reactive groups at the end that may react to covalently close the circle (e.g., thiols at both ends of the oligonucleotide could form an disulfide bond). Upon cleavage of all but one of the linkers connecting the FEs and complementing elements, a circular templated molecule is formed, attached to the template at one point.

D. Templating of linear, branched, circular and scrambled linear molecules by linear template.
(a) A linear templated molecule with the same sequence of FEs as obtained after incorporation, but before reaction, of the monomer building blocks. (b) A linear templated molecule with a scrambled sequence, i.e., the sequence of the FEs in the templated molecule does not correspond to the sequence obtained right after incorporation, but before reaction of the FEs. (c) A circular templated molecule obtained by pairwise reaction of the following FEs with each other: FE1/FE2, FE2/FE3, FE3/FE5, FE5/FE4, FE4/FE1. (d) A branched molecule obtained by pairwise reaction of the following functional entities with each other: FE1/FE2, FE2/FE3, FE2/FE4, and FE4/FE5. (e) A branched molecule obtained by pairwise reaction of the following functional entities with each other: FE1/FE2, FE2/FE4, FE2/FE5, FE2/FE3.

Figure 27. Non-equal number of reactive groups (X) and (Y). The number of reactive groups (X) can be higher than, equal to, or lower than the number of reactive groups (Y). When the number of (X) and (Y) are different, scrambling results. In the figure the scaffold (the molecular moiety to which the functional groups of the monomer building blocks become attached) is directly attached to the template. The

scaffold may also be part of a monomer building block (i.e., the functional entity of the monomer building block comprises a scaffold moiety, including reactive groups type II (Y).

(A). Number of encoded reactive groups X per template equals the number of reactive groups (Y) on the anchorage point (also called the scaffold).

(B). Number of encoded reactive groups X per template is less than the number of encodable substitutent positions Y on the scaffold. This leads to scrambling regarding which of the reactive groups (Y) on the scaffold (anchorage point) will react with an (X) on the monomer building blocks.

C. Number of encoded reactive groups X per template is larger than the number of reactive groups on the scaffold. This leads to scrambling regarding which of the reactive groups (Y) on the scaffold (anchorage point) will react with a reactive group (X) on the monomer building blocks.

Figure 28. Monomer building blocks.

(A) A monomer building block with one reactive group type II (X), connecting the functional group (Rx) with the complementing element. This type of monomer building block may be used for the simultaneous reaction and activation protocol (Figure ** .... **).

(B) A monomer building block with two reactive groups type II (X and Y), connecting the complementing element and the functional group (Rx).

(C) A monomer building block with one reactive group type II (X). The reactive group (X) does not link the functional group (Rx) and the complementing element, wherefore a linker (L) is needed for the activation step (in order to release the functional entity from the complementing element)

(D) A monomer building block with four reactive groups type II (Y). The four reactive groups and the functional group Rx may serve as a scaffold, onto which substituents (encoded by monomers complementinig the same template) are coupled through reaction of reactive groups (X) on these monomer building blocks with the reactive groups (Y) on this monomer building block. In this example, no cleavable linker is indicated. Therefore, after the templating reactions the templated molecule may be attached to the template through the linker of this monomer building block.

Figure 29. Templating involving simultaneous reaction and activation.

Templating using 4 monomer building blocks each with one reactive group type II (X), and an anchorage point carrying 4 reactive groups (Y). The reaction of X and Y involves simultaneous activation (cleavage) which releases X from the complementing element.

(A) The reactive groups type II (X) are of similar kind.

(B) The reactive groups type II (X1, X2, X3, X4) are of different kinds, i.e. the pairwise reactions between reactions X1/Y1, X2/Y2, X3/Y3, and X4/Y4 are orthogonal or partly orthogonal. For example, X1 preferably reacts with Y1, not Y2, Y3 or Y4. The anchorage point may be attached directly to the template, or to the complementing template. In case the anchorage point is attached to a complementing element, as a whole it is considered a monomer building block.

Figure 30. Reaction types allowing simultaneous reaction and activation. Different classes of reactions are shown which mediate translocation of a functional group from one monomer building block to another, or to an anchorage point. The reactions have been grouped into three different classes: Nucleophilic substitutions, addition-elimination reactions, and transition metal catalyzed reactions These reactions are compatible with simultaneous reaction and activation (as described in general terms in figure **C**).

(A) Reaction of nucleophiles with carbonyls. As a result of the nucleophilic substitution, the functional group R is translocated to the monomer building block initially carrying the nucleophile.

(B) Nucleophilic attack by the amine on the thioester leads to formation of an amide bond, in effect translocating the functional group R of the thioester to the other monomer building block.

(C) Reaction between hydrazine and β-ketoester leads to formation of pyrazolone, in effect translocating the R and R' functional groups to the other monomer building block.

(D) Reaction of hydroxylamine with β-ketoester leads to formation of the isoxazolone, thereby translocating the R and R' groups to the other monomer building block.

(E) Reaction of thiourea with β-ketoester leads to formation of the pyrimidine, thereby translocating the R and R' groups to the other monomer building block.

(F) Reaction of urea with malonate leads to formation of pyrimidine, thereby translocating the R group to the other monomer building block.

(G) Depending on whether Z = O or Z = NH, a Heck reaction followed by a nucleophilic substitution leads to formation of coumarin or quinolinon, thereby translocating the R and R' groups to the other monomer building block.

(H) Reaction of hydrazine and phthalimides leads to formation of phthalhydrazide, thereby translocating the R and R' groups to the other monomer building block.

(I) Reaction of amino acid esters leads to formation of diketopiperazine, thereby translocating the R group to the other monomer building block.

(J) Reaction of urea with α-substituted esters leads to formation of hydantoin, and translocation of the R and R' groups to the other monomer building block.

(K) Alkylation may be achieved by reaction of various nucleophiles with sulfonates. This translocates the functional groups R and R' to the other monomer building block.

(L) Reaction of a di-activated alkene containing an electron withdrawing and a leaving group, whereby the alkene is translocated to the nucleophile.

(M) Reaction of disulfide with mercaptane leads to formation of a disulfide, thereby translocating the R' group to the other monomer building block.

(N) Reaction of amino acid esters and amino ketones leads to formation of benzodiazepinone, thereby translocating the R group to the other monomer building block.

(O) Reaction of phosphonates with aldehydes or ketones leads to formation of substituted alkenes, thereby translocating the R'' group to the other monomer building block.

(P) Reaction of boronates with aryls or heteroaryls results in transfer of an aryl group to the other monomer building block (to form a biaryl).

(Q) Reaction arylsulfonates with boronates leads to transfer of the aryl group.

(R) Reaction of boronates with vinyls (or alkynes) results in transfer of an aryl group to the other monomer building block to form a vinylarene (or alkynylarene).

(S) Reaction between aliphatic boronates and arylhalides, whereby the alkyl group is translocated to yield an alkylarene.

(T) Transition metal catalysed alpha-alkylation through reaction between an enolether and an arylhallide, thereby translocating the aliphatic part.

(U) Condensations between e.g. enamines or enolethers with aldehydes leading to formation of alpha-hydroxy carbonyls or alpha,beta-unsaturated carbonyls. The reaction translocates the nucleophilic part.

(V) Alkylation of alkylhalides by e.g. enamines or enolethers. The reaction translocates the nucleophilic part.

(W) [2+4] cycloadditions, translocating the diene-part.

(X) [2+4] cycloadditions, translocating the ene-part.

(Y) [3+2] cycloadditions between azides and alkenes, leading to triazoles by translocation of the ene-part.

(Z) [3+2] cycloadditions between nitriloxides and alkenes, leading to isoxazoles by translocation of the ene-part.

Figure 31. Templating involving non-simultaneous reaction and activation: Reaction of reactive groups (type II), followed by cleavage of the linkers that connect functional entities with complementing elements.

Templating using 4 monomer building blocks each with one reactive group type II (X), and an anchorage point carrying 4 reactive groups (Y). The reaction of X and Y does not involve simultaneous activation (cleavage), wherefore the reaction of X and Y is followed by cleavage of the linker L, which releases the functional group Rx from the complementing element.

(A) The reactive groups type II (X) are of similar kind, i.e., they may react with the same type of reactive group (Y). (B) The reactive groups type II (X1, X2, X3, X4) are of different kinds, i.e. the reactions between X1/Y1, X2/Y2, X3/Y3, and X4/Y4 are orthogonal or partly orthogonal. For example, X1 preferably reacts with Y1, not Y2, Y3 or Y4. The anchorage point may be attached directly to the template, or to the complementing template. In case the anchorage point is attached to a complementing element, as a whole it is considered a monomer building block.

Figure 32. Pairs of reactive groups (X) and (Y), and the resulting bond (XY).

A collection of reactive groups that may be used for templated synthesis are shown, along with the bonds formed upon their reaction. After reaction, activation (cleavage) may be required (see Figure 31).

Figure 33. Anchorage sites for the templated molecule.

The templated molecule may be attached to the template that encodes it (A) through a linker that is connected directly to the template near the end of the template, or (B) through a linker that is connected directly to the template, at a more central position on the template, or (C) by way of a monomer building block carrying the anchorage point (a reactive group that becomes the linkage to the templated molecule).

Figure 34. Scrambling.

When the functional entities react after incorporation of the monomer building blocks, the position or sequence of functional groups in the templated molecule may not always be uniquely determined by the template sequence.

(1) The functional groups R1, R2, R3, and R4 may take any of the four positions on the scaffold molecule (i.e., the reactive group X of a monomer building block may react with any of the reactive groups Y on the anchorage point.

(2) The sequence of one arm of this branched molecule may be e.g. R5-R3-R2 (as shown), or R5-R2-R3 (not shown), or R5-R4-R3 (not shown), or any other of a number of possible sequences. Also, the identity of the functional group coupled to e.g. the left part of the molecule, may be ither of any of R1, R2, R3, or R4.

(3) As in (2), a number of possible sequences of functional groups are possible, in addition to the shown sequence R1-R2-R5-R4-R3.

(4) Here a non-scrambled templated molecule is shown, in which the sequence of the functional entitities when incorporated corresponds to the sequence of the templated molecule (R1-R2-R3-R4-R5). When desired, scrambling may be partly or fully avoided by directional encoding or the use of for example zipper boxes in the linkers (see figures 40, 44-47).

(5) As in (2) and (3), a number of possible sequences and positions of the functional entities are possible.

Figure 35. Monomer building blocks – examples of linker design.

Different designs of monomer building blocks are shown, used in various schemes of templating.

(A) The complementing element may be a oligonucleotide, to which a linker carrying the functional entity is attached to the central part of the oligonucleotide. The portion of the linker marked "a" may represent a nucleotide se-

quence to which a single stranded nucleotide may be annealed in order to make the linker more rigid.

(B) Both the complementing element and the linker may be made up of an oligonucleotide. The horizontal part here represents the complementing element, and the vertical part represents the linker. The linker may contain a sequence "a" that functions as a zipper box (see figure 40).

(C) - (I) Monomer building blocks used in the directional encoding schemes (see figure 44-47). The monomer building blocks of (F), (H) and (I) are initiators used to initiate the directional encoding process described in figures 44-47. " a " and " a' " represent complementary units (e.g. complementary nucleotide sequences) that anneal specifically to each other under certain conditions. "Nu" is a nucleophile that may react with an electrophile "E"

Figure 36 Coupling of functional entities to oligonucleotide-based monomer building blocks.

Reactions and reagents are shown that may be used for the coupling of functional entities to modified oligonucleotides (modified with thiol, carboxylic acid, halide, or amine), without significant reaction with the unmodified part of the oligonucleotide.

Figure 37 Oligonucleotide-based monomer building blocks. Examples of linker and functional entity (FE) design and synthesis.

Examples are shown where the complementing elements of the monomer building blocks comprises oligonucleotides of length e.g. 8-20 nucleotides (oligonucleotide is drawn as a thick black line). Part of or all of the oligonucleotide may comprise the complementing element. In the case where only part of the oligonucleotide represents the complementing element, the remaining portion of the oligonucleotide may constitute a linker (see also figure **...**). In the examples, a linker is attached to the base on the 3'- or 5'-end of the oligonucleotide. This linker may be attached on any nucleotide in the oligonucleotide sequence, and also, it may be attached to any molecular moiety on the oligonucleotide, as long as it does not abolish specific interaction of the complementing element with the template.

(A) A monomer building block in which the linker (L) connects the base of the terminal nucleotide with the functional entity.

(B) A monomer building block in which a polyethylene glycol (PEG) linker of between one and twenty ethylene glycol units connects the complementing

element with the functional entity which contains a nucleophile (a primary amine).

(C) A monomer building block in which a linker (L) connects the functional entity which contains an electrophile (an ester or thioester).

(D) A monomer building block comprising a Boc-protected amine (which may be deprotected with mild acid), and an ester. The deprotected amine may react with an ester of another monomer building block, to give an amide bond.

Figure 38. Oligonucleotide-based monomer building blocks. Example of complementing element design, allowing for high monomer diversity.

(A) Template carrying 6 coding elements (BOX 1-6), each containing a partly random sequence (X specifies either C or G), and a constant sequence that is identical for all sequences in the group (e.g., all BOX 1 sequences carry a central ATATTT sequence). By using C and G only (or, alternatively, A and T only), the individual sequences (e.g., the sequences belonging to the group of BOX 1 sequences), have almost identical annealing temperatures wherefore mis-annealing is insignificant. In the example, BOX 2 and BOX 3 are identical wherefore BOX 2 and BOX 3 may encode the same type of functional entities (comprising the same type of reactive groups of type II). The attachment point of the linker that connects the complementing element and the functional entity is not specified in the figure. Ideally, the linker is attached to a nucleotide in the constant region, in order to avoid bias in the annealing process.

(B) Example coding element sequences. Example BOX 1 and BOX 6 sequences are shown. The example BOX1 sequence represents one specific sequence out of 1024 different sequences that anneal specifically to the corresponding BOX 1 complementing elements; the example BOX 6 sequence represents one specific sequence out of 128 different sequences that anneal to the corresponding BOX 6 complementing elements.

(C) Templating using six monomers. Five classes of coding elements are used (BOX 2 and 3 are of the same class, i.e., the corresponding complementing elements of this class may anneal to both BOX 2 and 3). Reactive groups type II X and Y react; S and T react; A and B react; and C and D react. In the example the X/Y pair is orthogonal to S/T orthogonal to A/B orthogonal to C/D. Reaction of X with Y results in cleavage of R1 from the complementing element and translocation to R4. Reaction of S and T, followed by

cleavage of the linker L leads to translocation of R2 and R3 onto R4. Reaction of A with B, and C with D translocates R5 and R6 to R4. In this example, the functional entity of the monomer binding to BOX 4 serves as a "scaffold" onto which is added various substituents.

Figure 39 Use of rigid or partially rigid linkers to increase probability of reaction between the functional entities of the incorporated monomer building blocks.

(A) By using linkers comprising one or more flexible regions ("hinges") and one or more rigid regions, the probability of two functional entities getting into reactive contact may be increased.

(B) Symbol used for monomer building block with a rigid part and two flexible hinges.

(C) A monomer building block with the characteristics described in (B): The monomer building block contains an oligonucleotide as complementing element (horizontal line), and a oligonucleotide as linker connecting the functional entity (FE) with the complementing element. Annealing of a complementary sequence to the central part of the linker leads to formation of a rigid double helix; at either end of the linker a single-stranded region remains, which constitutes the two flexible hinges.

Figure 40 Use of zipper box to increase probability of reaction between the functional entities of the incorporated monomer building blocks.

(A) The linkers in this example carry zipper boxes (a) or (a'), that are complementary. By operating at a temperature that allows transient interaction of (a) and (a'), the reactive groups X and Y are brought into close proximity during multiple annealing events, which has the effect of keeping X and Y in close proximity in a larger fraction of the time than otherwise achievable. Alternatively, one may cycle the temperature between a low temperature (where the zipper boxes pairwise interacts stably), and a higher temperature (where the zipper boxes are apart, but where the complementing element remains stably attached to the coding element of the template). By cycling between the high and low temperature several times, a given reactive group X is exposed to several reactive groups Y, and eventually will react to form an XY bond.

(B) Sequences of two oligonucleotide-based monomer building blocks. The region constituting the complementing element, linker and zipper box is indicated.

Figure 41. Templated synthesis of organic compounds – examples.

(A) Three monomer building blocks are used. Each monomer building block comprises an activated ester (reactive group of type II, (X)) where the ester moiety carries a functional group Rx. Upon reaction between the esters and the amines on the scaffold (scaffold may be attached to the template), amide bonds are formed, and the Rx groups are now coupled to the scaffold via amide bonds. This is thus an example of simultaneous reaction (amide formation) and activation (release of the Rx moiety from the complementing elements), see figure 29.

(B) Analogously to (A), three amines react with three esters to form three amide bonds, thereby coupling the functional groups Rx to the scaffold moiety. However, as opposed to (A), the scaffold is here encoded by the template.

(C) Three monomer building blocks are used. The nucleophilic amine at the far right (part of the anchorage point) attacks the ester carbonyl of the third monomer; the amine of the third monomer attacks the thioester of the second monomer, and the Horner-Wittig Emmans reagent of the first monomer reacts with the aldehyde of the third monomer under alkaline conditions. This forms the templated molecule. The double bond may be post-templating modified by hydrogenation to form a saturated bond, or alternatively, submitted to a Michael addition.

(D) The thiol of the scaffold reacts with the pyridine-disulfide of monomer 1. The amine of the scaffold reacts with the ester of the second monomer. The double nitril activated alpha-position is acylated by the monomer 3's thioester in the presence of base. The aryliodide undergoes Suzuki coupling with the arylboronate of monomer 4 to yield the biaryl moiety.

(E) Monomer 1 acylates the primary amine. The aryliodide undergoes a Suzuki coupling by monomer 2 and the benzylic amine is acylated by monomer 3. Acylation of the hydrazine followed by cyclization leads to formation of an hydroxy-pyrazole. The arylbromide undergoes Suzuki coupling with the aryl boronate of monomer 1 and finally the aldehyde reactions with the Horner-Wittig-Emmons reagent of monomer 4 to yield an alpha, beta-unsaturated amide, which may be further

functionalized by either reduction with H_2/Pd-C or undergo Micael addition with nucleophiles.

Figure 42. α- and β-peptides, hydrazino peptides and peptoids. Encoding by use of oligonucleotide-based monomer building blocks.
It is shown how templated synthesis may be used to generate α- and β-peptides, hydrazino peptides and peptoids.

Figure 43. Templating of α–, β–, γ–, and ω-peptide through use of cyclic anhydrides
It is shown how templated synthesis may be used to generate α-, β-, g- and ω-peptides, through the use of cyclic anhydrides.

Figure 44. Directional encoding by hybridization and release.
Individual monomer building blocks are restricted in freedom to move by use of a complementary oligonucleotide region in the linker. Reactions are initiated by addition of an initiator monomer building block, which is not restricted in movement. The functional entity of the initiator reacts with the functional entity of monomer building block 1, translocating functional entity of initiator monomer building block to monomer building block 1. Following FE-transfer, hybridization between linkers (a-a', b-b' complementary regions) of initator and monomer1 relieves movement restrictions of monomer building block 1. Monomer building block 1 then approaches monomer building block 2, translocation of functional entities, linker hybridization, elimination of restriction in movement of monomer building block 2 and so forth. The intitiator may be present throughout the process and need not to be added separately. In such a case, as soon as monomer building block 1 has hybridized to the template, functional entities will be translocated from initiator monomer building block, monomer building block 1 released from restrictions in movement and then act as initiator once monomer building block 2 hybridizes.

Figure 44, Example 1. Directional polymer encoding - peptide (α, β), peptoid or hydrazine peptide.

Figure 45. Directional encoding by nucleation of hybridization and release.

Individual monomer building blocks are restricted in freedom to move by use of a complementary oligonucleotide region in the linker. Reactions are initiated by addition of an initiator monomer building block, which is not restricted in movement. Reactions are initiated by hybridization between linker regions c-c' between initiator monomer building block and monomer building block1. This allows functional entities to be held in close proximity. The functional entity of the initiator monomer building block reacts with the functional entity of monomer building block 1, translocating functional entity of initiator to monomer1. Following FE-transfer, hybridization between linkers (a-a', b-b' complementary regions) of initator and monomer building block 1, relieves movement restrictions of monomer building block 1. Monomer building block 1 then approaches monomer building block 2, translocation of functional entities, linker hybridization, elimination of restriction in movement of monomer building block 2 and so forth. The intitiator may be present throughout the process and need not to be added separately. In such a case, as soon as monomer building block 1 has hybridized, functional entities will be translocated from initiator monomer building block, monomer building block 1 released from restrictions in movement and then act as initiator once monomer building block 2 hybridizes.

Figure 46 Directional encoding by nucleophilic attack and release

Individual monomer building blocks are restricted in freedom to move by use of a cleavable linker. Reactions are initiated by addition of an initiater monomer building block, which is not restricted in movement. The functional entity of the initiator reacts with the functional entity of monomer building block 1, translocating functional entity of initiator to monomer1. Following FE-transfer, a nucleophile will open the linker, which relieves movement restrictions of monomer building block 1. Monomer building block 1 then approaches monomer2, translocation of functional entities, linker cleavage, elimination of restriction in movement of monomer building block 2 and so forth. The intitiator may be present throughout the process and need not to be added separately. In such a case, as soon as monomer building block 1 has hybridized to the template, functional entities will be translocated from initiator monomer building block, monomer building block 1 released from restrictions in movement and then act as initiator once monomer building block 2 hybridizes.

Figure 46, example 1. Directional encoding by nucleophilic attack and release

Example A. General principle, example B. Using a regid setup to avoid self cleavage of the linker by a nucleophilic functional entity, example C. As B. using a more nucleophilic group for cleaving the linker.

Figure 47. Directional encoding by release mediated by induced nucleophilic attack

Individual monomer building blocks are restricted in freedom to move by use of a cleavable linker. Reactions are initiated by addition of an initiater monomer building block, which is not restricted in movement. The functional entity of the initiator reacts with the functional entity of monomer building block 1, translocating functional entity of initiator to monomer building block 1. Following FE-transfer, a nucleophile will be generated which opens the linker, thereby relieving movement restrictions of monomer building block 1. Monomer building block 1 then approaches monomer2, translocation of functional entities, formation of a nucleophile, linker cleavage by nucleophile, elimination of restriction in movement of monomer building block 2 and so forth. The intitiator may be present throughout the process and need not to be added separately. In such a case, as soon as monomer building block 1 has hybridized to the template, functional entities will be translocated from initiator monomer building block, monomer building block 1 released from restrictions in movement and then act as initiator once monomer building block 2 hybridizes.

Figure 47, example 1. Directional encoding by induced nucleophilic attack and release.

Example A and B. Generation of a nucleophile by function entity translocation.

Figure 47, example 2. Directional encoding by induced nucleophilic attack and release - peptide (α, β), peptoid or hydrazinopeptide.

Figure 48. Multi-step templating.

By the addition of the monomer building blocks in separate steps, the scrambling effect may be decreased.

Figure 49. Generation of new reactive groups upon reaction of the reactive groups X and Y.

In cases where the reaction of X and Y leads to formation of a new reactive group Z, this may be exploited to increase the diversity of the templated molecule, by incorporating monomer building blocks carrying reactive groups Q that react with Z.
(A) X abd Y react to form Z, which in itself does lead to release from the complementing element. Upon reaction of Z with Q, and cleavage of the linker that connects Z to the complementing element, the templated molecule is formed.
(B) In this case, reaction of X and Y to form Z simultaneously cleaves the linker connecting X to the complementing element. Upon reaction of Z with Q, the templated molecule is formed.
(C) X and Y react to form Z, coupled to R5. The additional monomer building blocksare added. These monomer building blocks are complementary to the linker portion of the monomer building blocks that were added first. Asa result, Z and Q react to form the templated molecule.

Figure 49, example 1. Templated synthesis by generating a new reactive group.
The reaction of the functional entities of the first three monomer building blocks leads to formation of two double bonds, which may react with two hydroxylamines carried in by the monomer building blocks added in the second step, and leads to formation of an ester, which may react with the an hydroxylamine, carried in by the monomer added in the second step. Finally, the linkers are cleaved, generating the templated molecule.

Figure 50. Linkers that may be used to tether the templated molecule to the the template after reaction.

Figure 51. Cleavable linkers.
Cleavable linkers, the conditions for their cleavage, and the resulting products are shown.

Figure 52. Templated molecules - examples.

Figure 53. Post-templating modification of templated molecule.

After the templating process has been performed, the templated molecules may be modified to introduce new characteristics. This list describes some of these post-templating modificiations.

Definitions

α-peptide: Peptide comprising or essentially consisting of at least two α-amino acids linked to one another by a linker including a peptide bond.

Amino acid: Entity comprising an amino terminal part (NH_2) and a carboxy terminal part (COOH) separated by a central part comprising a carbon atom, or a chain of carbon atoms, comprising at least one side chain or functional group. NH_2 refers to the amino group present at the amino terminal end of an amino acid or peptide, and COOH refers to the carboxy group present at the carboxy terminal end of an amino acid or peptide. The generic term amino acid comprises both natural and non-natural amino acids. Natural amino acids of standard nomenclature as listed in J. Biol. Chem., 243:3552-59 (1969) and adopted in 37 C.F.R., section 1.822(b)(2) belong to the group of amino acids listed in Table 2 herein below. Non-natural amino acids are those not listed in Table 2. Examples of non-natural amino acids are those listed e.g. in 37 C.F.R. section 1.822(b)(4), all of which are incorporated herein by reference. Further examples of non-natural amino acids are listed herein below. Amino acid residues described herein can be in the "D" or or "L" isomeric form.

Symbols		Amino acid
1-Letter	3-Letter	
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine

T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
C	Cys	cysteine

Table 2. Natural amino acids and their respective codes.

Amino acid precursor: Moiety capable of generating an amino acid residue following incorporation of the precursor into a peptide.

Amplifying: Any process or combination of process steps that increases the number of copies of a templated molecule. Amplification of templated molecules may be carried out by any state of the art method including, but not limited to, a polymerase chain reaction to increase the copy number of each template, and using the templates for synthesising additional copies of the templated molecules comprising a sequence of functional groups resulting from the synthesis of the templated molecule being templated by the template. Any amplification reaction or combination of such reactions known in the art can be used as appropriate as readily recognized by those skilled in the art. Accordingly, templated molecules can be amplified by using the polymerase chain reaction (PCR), ligase chain reaction (LCR), in vivo amplification of cloned DNA, and the like. The amplification method should preferably result in the proportions of the amplified mixture being essentially representative of the proportions of templates of different sequences in a mixture prior to amplification.

Base: Nitrogeneous base moiety of a natural or non-natural nucleotide, or a derivative of such a nucleotide comprising alternative sugar or phosphate moieties. Base moieties include any moiety that is different from a naturally occurring moiety and

capable of complementing one or more bases of the opposite nucleotide strad of a double helix.

Building block: Species comprising a) at least one complementing element comprising at least one recognition group capable of recognising a predetermined coding element, b) at least one functional entity comprising a functional group and a reactive group, and c) at least one linker separating the at least one functional entity from the at least one complementing element, wherein the building block does not comprise a ribosome. Preferred building blocks are capable of being incorporated into a nucleotide strand and/or capable of being linked by reactions involving reactive groups of type I and/or type II as described herein.

Cleavable linker: Residue or bond capable of being cleaved under predetermined conditions.

Cleaving: Breaking a chemical bond. The bond may be a covalent bond or a non-covalent bond.

Coding element: Element of a template comprising a recognition group and capable of recognising a predetermined complementing element of a building block. The recognition may result from the formation of a covalent bond or from the formation of a non-covalent bond between corresponding pairs of coding elements and complementing elements capable of interacting with one another.

Coding element complementation: Contacting a coding element with a predetermined complementing element capable of recognising said coding element.

Complementing: Process of bringing a coding element into reactive contact with a predetermined complementing element capable of recognising said coding element. When the coding element and the complement element comprises a natural nucleotide comprising a base moiety, predetermined sets of nucleotides are capable of complementing each other by means of hydrogen bonds formed between the base moieties.

Complementing element: Element of a building block. Linked to at least one functional entity by means of a linker. See coding element.

Complementing template: A sequence of complementing elements, wherein each complementing element is covalently linked to a neighbouring complementing element. A complementing element is capable of recognising a predetermined coding element. The complementing template may be linear or branched.

Complex: Templated molecule linked to the template that templated the synthesis of the templated molecule. The template can be a complementing template as defined herein that is optionally hybridised or otherwise attached to a corresponding template of linked coding elements.

Contacting: Bringing e.g. corresponding reactive groups or corresponding binding partners or hybridization partners into reactive contact with each other. The reactive contact is evident from a reaction or the formation of a bond or a hybridization between the partners.

Corresponding binding partners: Binding partners capable of reacting with each other.

Corresponding reactive groups: Reactive groups capable of reacting with each other.

Functional entity: Entity forming part of a building block. The functional entity comprises a functional group and a reactive group capable of linking neighbouring, functional groups.

Functional group: Group forming part of a templated molecule. The sequence of functional groups in a templated molecule is a result of the capability of the template to template the synthesis of the templated molecule.

Interacting: Used interchangably with contacting. Bringing species such as e.g. correspnding binding partners in the form of e.g. coding elements and complementing elements into reactive contact with each other. The reaction may be

mediated by recognition groups forming corresponding binding partners by means of covalent or non-covalent bonds. The interaction may occur as a result of mixing a template comprising a plurality of coding elements with a plurality of building blocks.

Ligand: Used herein to describe a templated molecule capable of targeting a target molecule. In a population of candidate template molecules, a ligand is one which binds with greater affinity than that of the bulk population. In a candidate mixture there can exist more than one ligand for a given target. The ligands can differ from one another in their binding affinities for the target molecule.

Linker: A residue or chemical bond separating at least two species. The species may be retained at an essentially fixed distance, or the linker may be flexible and allow the species some freedom of movement in relation to each other. The link can be a covalent bond or a non-covalent bond. Linked species include e.g. a complementing element and a functional entity of a building block, neighbouring coding elements of a template, neighbouring complementing elements of a complementing template, and neighbouring functional groups of a templated molecule.

Natural nucleotide: Any of the four deoxyribonucleotides, dA, dG, dT, and dC (constituents of DNA), and the four ribonucleotides, A, G, U, and C (constituents of RNA) are the natural nucleotides. Each natural nucleotide comprises or essentially consists of a sugar moiety (ribose or deoxyribose), a phosphate moiety, and a natural/standard base moiety. Natural nucleotides bind to complementary nucleotides according to well-known rules of base pairing (Watson and Crick, Nature ????), where adenine (A) pairs with thymine (T) or uracil (U); and where guanine (G) pairs with cytosine (C), wherein corresponding base-pairs are part of complementary, anti-parallel nucleotide strands. The base pairing results in a specific hybridization between predetermined and complementary nucleotides. The base pairing is the basis by which enzymes are able to catalyze the synthesis of an oligonucleotide complementary to the template oligonucleotide. In this synthesis, building blocks (normally the triphosphates of ribo or deoxyribo derivatives of A, T, U, C, or G) are directed by a template oligonucleotide to form a complementary oligonucleotide with the correct, complementary sequence. The recognition of an oligonucleotide sequence by its complementary sequence is mediated by corresponding and interact-

ing bases forming base pairs. In nature, the specific interactions leading to base pairing are governed by the size of the bases and the pattern of hydrogen bond donors and acceptors of the bases. A large purine base (A or G) pairs with a small pyrimidine base (T, U or C). Additionally, base pair recognition between bases is influenced by hydrogen bonds formed between the bases. In the geometry of the Watson-Crick base pair, a six membered ring (a pyrimidine in natural oligonucleotides) is juxtaposed to a ring system composed of a fused, six membered ring and a five membered ring (a purine in natural oligonucleotides), with a middle hydrogen bond linking two ring atoms, and hydrogen bonds on either side joining functional groups appended to each of the rings, with donor groups paired with acceptor groups.

Neighbouring: Elements, groups, entities or residues located next to one another in a sequence are said to be neighbouring. In cases where two complementing elements, each linked to a functional entity, are linked to one another through one (or more) complementing element(s) that is not linked to a functional entity, the aforementioned complementing elements are said to be neighbouring and said two complementing elements define neighbouring functional entities and neighbouring coding elements that can be linked to one another, either directly or through one (or more) coding element(s).

Non-natural amino acid: Any amino acid not included in Table 2 herein above. Non-natural amino acids includes, but is not limited to modified amino acids, L-amino acids, and stereoisomers of D-amino acids.

Non-natural base pairing: Base pairing among non-natural nucleotides, or among a natural nucleotide and a non-natural nucleotide. Examples are described in US 6,037,120, wherein eight non-standard nucleotides are described, and wherein the natural base has been replaced by a non-natural base. As is the case for natural nucleotides, the non-natural base pairs involve a monocyclic, six membered ring pairing with a fused, bicyclic heterocyclic ring system composed of a five member ring fused with a six membered ring. However, the patterns of hydrogen bonds through which the base pairing is established are different from those found in the natural AT, AU and GC base pairs. In this expanded set of base pairs obeying the Watson-Crick hydrogen-bonding rules, A pairs with T (or U), G pairs with C, iso-C

pairs with iso-G, and K pairs with X, H pairs with J, and M pairs with N (Figure 2). Nucleobases capable of base pairing without obeying Watson-Crick hydrogen-bonding rules have also been described (Berger et al., 2000, Nucleic Acids Research, 28, pp. 2911-2914).

Non-natural nucleotide: Any nucleotide not falling within the definition of a natural nucleotide.

Nucleotide: Nucleotides as used herein refers to both natural nucleotides and non-natural nucleotides capable of being incorporated - in a template-directed manner - into an oligonucleotide, preferably by means of an enzyme comprising DNA or RNA dependent DNA or RNA polymerase activity, including variants and functional equivalents of natural or recombinant DNA or RNA polymerases. Corresponding binding partners in the form of coding elements and complementing elements comprising a nucleotide part are capable of interacting with each other by means of hydrogen bonds. The interaction is generally termed "base-pairing". Nucleotides may differ from natural nucleotides by having a different phosphate moiety, sugar moiety and/or base moiety. Nucleotides may accordingly be bound to their respective neighbour(s) in a template or a complementing template by a natural bond in the form of a phosphodiester bond, or in the form of a non-natural bond, such as e.g. a peptide bond as in the case of PNA (peptide nucleic acids).

Nucleotide analog: Nucleotide capable of base-pairing with another nucleotide, but incapable of being incorporated enzymatically into a template or a complementary template. Nucleotide analogs often includes monomers or oligomers containing non-natural bases or non-natural backbone structures that do not facilitate incorporation into an oligonucleotide in a template-directed manner. However, interaction with other monomers and/or oligomers through specific base pairing is possible. Alternative oligomers capable of specifically base pairing, but unable to serve as a substrate of enzymes, such as DNA polymerases and RNA polymerases, or mutants or functional equivalents thereof, are defined as nucleotide analogs herein. Oligonucleotide analogs includes e.g. nucleotides in which the phosphodiester-sugar backbone of natural oligonucleotides has been replaced with an alternative backbone include peptide nucleic acid (PNA), locked nucleic acid (LNA), and morpholinos.

Nucleotide derivative: Nucleotide or nucleotide analog further comprising an appended molecular entity. Often, derivatized building blocks (nucleotides to which a molecular entity have been appended) can be enzymatically incorporated into oligonucleotides by RNA or DNA polymerases, using as substrate the triphosphate of the derivatized nucleoside. In many cases such derivatized nucleotides are incorporated into the growing oligonucleotide chain with high specificity, meaning that the derivative is inserted opposite a predetermined nucleotide in the template. Such an incorporation will be understood to be a specific incorporation. The nucleotides can be derivatized on the bases, the ribose/deoxyribose unit, or on the phosphate. Preferred sites of derivatization on the bases include the 8-position of adenine, the 5-position of uracil, the 5- or 6-position of cytosine, and the 7-position of guanine. The nucleotide-analogs described below may be derivatized at the corresponding positions (Benner, United States Patent 6,037,120). Other sites of derivatization may be used, as long as the derivatization does not disrupt base pairing specificity. Preferred sites of derivatization on the ribose or deoxyribose moieties are the 5', 4' or 2' positions. In certain cases it may be desirable to stabilize the nucleic acids towards degradation, and it may be advantageous to use 2'-modified nucleotides (US patent 5,958,691). Again, other sites may be employed, as long as the base pairing specificity is not disrupted. Finally, the phosphates may be derivatized. Preferred derivatizations are phosphorothiote. Nucleotide analogs (as described below) may be derivatized similarly to nucleotides. It is clear that the various types of modifications mentioned herein above, including i) derivatization and ii) substitution of the natural bases or natural backbone structures with non-natural bases and alternative, non-natural backbone structures, respectively, can be applied once or more than once within the same molecule.

Oligonucleotide: Used herein interchangebly with polynucleotide. The term oligonucleotide comprises oligonucleotides of both natural and/or non-natural nucleotides, including any combination thereof. The natural and/or non-natural nucleotides may be linked by natural phosphodiester bonds or by non-natural bonds. Oligonucleotide is used interchancably with polynucleotide.

Oligomer: Molecule comprising a plurality of monomers that may be identical, of the same type, or different. Oligomer is used synonymously with polymer in order to describe any molecule comprising more than two monomers. Oligomers may be

homooligomers comprising a plurality of identical monomers, oligomers comprising different monomers of the same type, or heterooligomers comprising different types of monomers, wherein each type of monomer may be identical or different.

Partitioning: Process whereby templated molecules, or complexes comprising such molecules linked to a template, are preferentially bound to a target molecule and separated from templated molecules, or complexes comprising such molecules linked to a template, that do not have an affinity for - and is consequently not bound to - such target molecules. Partitioning can be accomplished by various methods known in the art. The only requirement is a means for separating targeted, templated molecules bound to a target molecule from templated molecules not bound to target molecules. The choice of partitioning method will depend on properties of the target molecule and of the templated molecule and can be made according to principles and properties known to those of ordinary skill in the art.

Peptide: Plurality of covalently linked amino acid residues defining a sequence and linked by amide bonds. The term is used analogously with oligopeptide and polypeptide. The amino acids may be both natural amino acids and non-natural amino acids, including any combination thereof. The natural and/or non-natural amino acids may be linked by peptide bonds or by non-peptide bonds. The term peptide also embraces post-translational modifications introduced by chemical or enzyme-catalyzed reactions, as are known in the art. Such post-translational modifications can be introduced prior to partitioning, if desired. Amino acids as specified herein will preferentially be in the L-stereoisomeric form. Amino acid analogs can be employed instead of the 20 naturally-occurring amino acids. Several such analogs are known, including fluorophenylalanine, norleucine, azetidine-2-carboxylic acid, S-aminoethyl cysteine, 4-methyl tryptophan and the like.

Plurality: At least two.

Polymer: Templated molecule characterised by a sequence of covalently linked residues each comprising a functional group, including H. Polymers according to the invention comprise at least two residues.

Polynucleotide: See oligonucleotide,

Precursor: Moiety comprising a residue and being capable of undergoing a reaction during template directed synthesis of a templated molecule, wherein the residue part of the precursor is built into the templated molecule.

Reactive group: Corresponding reactive groups being brought into reactive contact with each other are capable of forming a chemical bond linking e.g. a coding element and its complementing element, or coupling functional groups of a templated molecule.

Recognition group: Part of a coding element and involved in the recognition of the complementing element capable of recognising the coding element. Preferred recognition groups are natural and non-natural nitrogeneous bases of a natural or non-natural nucleotide.

Recombine: A recombination process recombines two or more sequences by a process, the product of which is a sequence comprising sequences from each of the two or more sequences. When involving nucleotides, the recombination involves an exchange of nucleotide sequences between two or more nucleotide molecules at sites of identical nucleotide sequences, or at sites of nucleotide sequences that are not identical, in which case the recombination can occur randomly. One type of recombination among nucleotide sequences is referred to in the art as gene shuffling.

Repetitive sequence: Sequence of at least two elements, groups, or residues, occurring more than once in a molecule.

Residue: A polymer comprises a sequence of covalently linked residues, wherein each residue comprises a functional group.

Ribose derivative: Ribose moiety forming part of a nucleoside capable of being enzymatically incorporated into a template or complementing template. Examples include e.g. derivatives distinguishing the ribose derivative from the riboses of natural ribonucleosides, including adenosine (A), guanosine (G), uridine (U) and cytidine (C). Further examples of ribose derivatives are described in e.g. US 5,786,461. The

term covers derivatives of deoxyriboses, and analogously with the above-mentioned disclosure, derivatives in this case distinguishes the deoxyribose derivative from the deoxyriboses of natural deoxyribonucleosides, including deoxyadenosine (dA), deoxyguanosine (dG), deoxythymidine (dT) and deoxycytidine (dC).

Selectively cleavable linker: Selectively cleavable linkers are not cleavable under conditions wherein a cleavable linker is cleaved. Accordingly, it is possible to cleave the cleavable linkers linking complementing elements and functional groups in a templated molecule without at the same time cleaving selectively cleavable linkers linking - in the same templated molecule - a subset of complementing elements and functional groups. It is thus possible to obtain a complex comprising a templated molecule and the template that has directed the template-mediated synthesis of the templated molecule, wherein the template and the templated molecule are linked by one or more, preferably one, selectively cleavable linker(s).

Specific recognition: The interaction of e.g. a coding element with preferably one predetermined complementing element. A specific recognition occurs when the affinity of a coding element recognition group for a complementing group results in the formation of predominantly only one type of corresponding binding partners. Simple mis-match incorporation does not exclude a specific recognition of corresponding binding partners. Specific recognition is a term which is defined on a case-by-case basis. In the context of a given interaction between predetermined binding partners, e.g. a templated molecule and a target molecule, a binding interaction of templated molecule and target molecule of a higher affinity than that measured between the target molecule and a candidate template molecule mixture is observed. In order to compare binding affinities, the conditions of both binding reactions must be essentially similar and preferably the same, and the conditions should be comparable to the conditions of the intended use. For the most accurate comparisons, measurements will be made that reflect the interaction between templated molecule as a whole and target as a whole. The templated molecules of the invention can be selected to be as specific as required, either by establishing selection conditions that demand a requisite specificity, or by tailoring and modifying the templated molecules.

Subunit: Monomer of coding element comprising at least one such subunit.

Support: Solid or semi-solid member to which e.g. coding elements can be attached during interaction with at least one complementing element of a building block. Functional molecules or target molecules may also be attached to a solid support during targeting. Examples of supports includes planar surfaces including silicon wafers as well as beads.

Tag: Entity capable of identifying a compound to which it is associated.

Target molecule: Any compound of interest for which a templated molecule in the form of a ligand is desired. A target molecule can be a protein, fusion protein, peptide, enzyme, nucleic acid, nucleic acid binding protein, carbohydrate, polysaccharide, glycoprotein, hormone, receptor, receptor ligand, cell membrane component, antigen, antibody, virus, virus component, substrate, metabolite, transition state analog, cofactor, inhibitor, drug, controlled substance, dye, nutrient, growth factor, toxin, lipid, glycolipid, etc., without limitation.

Template: Template refers to both a template of coding elements and a (complementing) template of complementing elements unless otherwise specified. When referring to a template of coding elements, each coding element is covalently linked to a neighbouring coding element. Each coding element is capable of recognising a predetermined complementing element. The template may be linear or branched. A template of coding elements actively takes part in the synthesis of the templated molecule, and the templating activity involves the formation of specific pairing partners in the form of coding element:complementing element hybrids, wherein the complementing element forms part of a building block also comprising the functional group forming part of the templated molecule. The template is preferably a string of nucleotides or nucleotide analogs. When the template comprises a string of nucleotides, the nucleotides may be natural or non-natural, and may be linked by e.g. phosphorothioate bonds or natural phosphodiester bonds. Nucleotide analogs may be linked e.g. by amide bonds, peptide bonds, or any equivalent means capable of linking nucleotide analogs so as to allow the nucleotide analog string to hybridize specifically with another string of nucleotides or nucleotide analogs. The sugar moiety of a nucleotide or nucleotide analog may be a ribose or a deoxyribose, a ribose derivative, or any other molecular moiety that allows the

template or complementing template to hybridise specifically to another string of nucleotides or nucleotide analogs.

Template directed synthesis: Used synonymously with template directed incorporation and templated synthesis. Template directed synthesis is the process, wherein the formation of a templated molecule comprising a sequence of covalently linked, functional groups involves contacting a string of coding elements with particular complementing elements. The process thus defines a one-to-one relationship between coding elements and functional groups, and the contacted coding element of the template directs the incorporation of the functional group into the templated molecule comprising a sequence of covalently linked, functional groups. Accordingly, there is a predetermined one to one relationship between the sequence of functional groups of the templated molecule and the sequence of coding elements of the template that templated the synthesis of the templated molecule. Thus, during the templated synthesis of the templated molecule, a functional group is initially contacting - by means of a linker moiety and/or a complementing element, or otherwise - the coding element capable of templating that particular functional group into the templated molecule. When the template comprises or essentially consists of nucleotides, a template directed synthesis of an oligonucleotide is based on an interaction of each nucleotide with its pairing partner in the template in a one-base-to-one-base pairing manner. The interaction specifies the incorporation of complementing nucleotides opposite their base pairing partners in the template. Consequently, one base, including a heterocyclic base, from each oligonucleotide strand interact when forming specific base-pairs. This base pairing specificity may be achieved through Watson-Crick hydrogen-bonding interactions between the bases, where the bases may be natural (i.e. A, T, G, C, U), and/or non-natural bases such as those e.g. disclosed e.g. in US 6,037,120, incorporated herein by reference. Further examples of non-natural bases are e.g. PNA (peptide nucleic acid), LNA (lock nucleic acid) and morpholinos. Base pairing of oligonucleotides containing non-standard base pairs can be achieved by other means than hydrogen bonding (e.g. interaction between hydrophobic nucleobases with "complementary" structures; Berger et al., 2000, Nucleic Acids Research, 28, pp. 2911-2914). The interacting oligonucleotide strands as well as the individual nucleotides are said to be complementary. The specificity of the interaction between oligomers results from the specific base pairing of a nucleotide with

another nucleotide or a predetermined subset of nucleotides, for example A base pairing with U, and C base pairing with G.

Templated: Feature of the templated molecule of the complex comprising a template linked to the templated molecule, wherein the templated molecule is obtainable by template directed synthesis using the template. Thus, one component of the complex (the template) is capable of templating the synthesis of the other component (the templated molecule). The term is also used to describe the synthesis of the templated molecule that involves the incorporation into the templated molecule of functional groups, wherein the incorporation of each functional group involves contacting a coding element with a particular functional group, or with a building block comprising said functional group, wherein the contacted coding element of the template directs the incorporation of functional groups into the templated molecule linked to the template that templated in this way the synthesis of the templated molecule. Thus, during the templated synthesis of the templated molecule, a functional group is initially contacting - either directly or by means of a linker moiety and/or a complementing element - the coding element capable of templating that particular functional group into the templated molecule.

Templated molecule: Molecule comprising a sequence of covalently linked. functional groups, wherein the templated molecule is obtainable by template directed synthesis using the template. Thus, one component of the complex (the template) is capable of templating the synthesis of the other component (the templated molecule). When the template comprises or essentially consists of nucleotides, the template is capable of being amplified, wherein said template amplification results in a plurality of templated molecules, wherein each templated molecule is generated by template directed synthesis using the template. Following amplification of a template, or a complementing template, templated molecules can be generated by a template directed synthesis using either a template of coding elements or a complementing template of complementing elements as a template for the template directed synthesis of the templated molecule.

Templating: Process of generating a templated molecule.

Variant: Template or templated molecule exhibiting a certain degree of identity or homology to a predetermined template or templated molecule, respectively.

Detailed Description of the Invention

In one preferred embodiment of the present invention, there is provided a "chemical display of templated molecules" which enables the generation of a huge number of "templated polymers" (e.g. from about 10^3 to about or more than e.g. 10^{18} as described elsewhere herein), wherein each templated molecule is individually linked to a "template" that serves as identification of that individual polymer (its sequence of residues) , as well as a means for amplification (many copies of the molecule can be prepared by a process that replicates the template). Preferred embodiments of the invention are disclosed in Figure 1 illustrating various steps of the method of the invention.

Step 1. Synthesis

Different monomer building blocks are synthesized. Building blocks comprise a functional entity and a complementing element that are linked by means of a cleavable linker (Figures 3 and 4). Preferred building blocks comprise a nucleotide to which have been appended a functional entity through a cleavable linker, and where the functional entity comprises or essentially consists of an "activatable" polymer unit (Figure 6).

Step 2. Incorporation

The building blocks are used as substrates in a template-dependent polymer synthesis. In one embodiment, the building blocks are nucleotide-derivatives and a polymerase is preferably used to incorporate the nucleotide-derivatives into an oligonucleotide strand according to the directions of a oligonucleotide template. As a result, a complementing template (a string of incorporated building blocks) is formed, from which the functional entities protrude. The sequence of functional entities is determined by the sequence of coding elements, such as nucleotides, of the template.

Figure 1 describes the use of a building block that carries the selectively cleavable linker which, after polymerization and activation, is capable of linking the templated

polymer to its template. Alternatively, the selectively cleavable linker can be comprised by an oligo capable of annealing upstream or downstream of the polymer-encoding portion of the template (see for example Figure 7 or 8), or the link could be to the template directly.

The building block can preferably be incorporated by an enzyme, such as for example DNA polymerase, RNA polymerase, Reverse Transcriptase, DNA ligase, RNA ligase, Taq DNA polymerase, HIV-1 Reverse Transcriptase, Klenow fragment, or any other enzyme that will catalyze the incorporation of complementing elements such as mono-, di- or polynucleotides. In some of these cases, a primer is required (for example DNA polymerase). In other cases, no primer is required (e.g., RNA polymerase).

Step 3. Polymerization

Each functional entity has preferably reacted with neighbouring functional entities to form a polymer during or after formation of the complementing template. A change in conditions, e.g., photolysis, change in temperature, or change in pH, may initiate the polymerisation either during or after complementing template formation.

Step 4. Activation

The formed polymer is preferably released from the complementing elements by cleavage of at least one linker, or a plurality of cleavable linkers, except at one or more predetermined position(s), including a single position, where the linker is not cleavable under conditions resulting in cleavage of the remaining linkers. The result is a templated polymer attached at one or more positions, preferably only at one position, to the template that encodes it.

Step 5. Selection and amplification

A selection process can subsequently be performed, wherein a huge number of different templated molecules, each attached to the template that directed its synthesis, is challenged with a molecular or physical target (e.g. a biological receptor or a surface), or is exposed to a certain screen. Templated molecules having desired characteristics (e.g., binding to a receptor) are recovered and amplified, by first amplifying the templates, and then using the templates for a new round of templated polymer synthesis. The process of selection and amplification can be repeated sev-

eral times, until a polymer with appropriate characteristics (e.g., high affinity for the receptor) is isolated.

A typical selection protocol involves the addition of a population (a library) of template-templated molecule complexes to an affinity column, to which a certain molecular target (e.g., a receptor) had been immobilized. After washing the column, the binders are eluted. This eluate consists of an enriched population of template-templated molecule complexes with affinity for the immobilized target molecule. The enriched population may be taken through an amplification round, and then be subjected to yet a selection round, where the conditions optionally may be more stringent. After a number of such selection-and-amplification rounds, an enriched population of high affine binders are obtained.

When selecting for the ability of a templated molecule to become internalized into a cell, the selection step may involve a simple mixing of the population of template-templated molecule complexes with cells. After incubation (to allow the internalization of the template-templated molecule complexes), the cells are washed, and the internalized template-templated molecule complexes may be recovered by lysis of the cells. As above, the template-templated molecule complexes may be amplified and taken through further rounds of selection-and-amplification. After a number of selection-and-amplification rounds, an enriched population of templated molecules with the ability to internalize are obtained.

Building blocks – molecular design

The building blocks (also termed "monomers") is preferably of the general design shown in Figure 3 and 4. The monomer in one embodiment comprises the following elements: Complementing element-Linker-Backbone comprising reactive group(s) type II-Functional group, where the complementing element comprises or essentially consists of a recognition group and reactive group(s) type I. In this case the linker is preferably a "traceless linker", i.e., a linker that does not leave any (undesirable) molecular entity on the functional entity. Building blocks with this composition are used in for example (Figure 15, example 7).

Alternatively, the monomer may have the composition Complementing element-Linker-Functional Group-Backbone containing reactive group(s) type II, in which case the desired functional group is created as a result of cleavage of the linker. Building blocks with this composition are used in for example (Figure 16, example 1).

The functional groups must be compatible with the desired method for incorporation of complementing elements, their polymerization and activation. Obviously, it is important to preserve the integrity of the template and the templated molecule in these processes.

Functional groups that are not compatible with the conditions of incorporation, polymerization or activation must be protected during these processes, or alternatively, the functional groups must be introduced after these processes have taken place. The latter is done by templating a functional group (e.g., an activated disulfide) that is compatible with the incorporation, polymerization and activation, and that will specifically react with a bifunctional molecule (e.g., a thiol connected to the desirable functional group, R_x), added after activation. Alternatively, functionalities may be introduced by e.g. oxidation, or any other form of treatment, of the incorporated functional entities after activation. In this way, functionalities such as components of natural effector molecules or synthetic drugs that are otherwise difficult to handle, may be incorporated. (Figure 24) provides examples of functional groups.

In some embodiments of the process of the invention as described herein, there is no need for a cleavable linker, as the polymerisation reaction involves cleavage of the linker (Figure 14 and Figure 14, example 1).

When being nucleotides, the complementing elements may contain one, two or several nucleotides or nucleotide-analogs. The use of di-, tri- or longer oligonucleotides presents a number of advantages. First, a higher monomer diversity may be encoded by the template. Second, the requirements for the site of attachment of the functional entity to the complementing element becomes more relaxed. Third, there would be less bulk per mononucleotide in the formed polynucleotide, potentially leading to higher display-efficiencies. Fourth, it would allow the display of polymers

with longer residue-unit-length. Also, it would allow the display of bigger functional groups.

In cases where a polymerase is employed for the incorporation of nucleotide comprising building blocks, it is preferred that the nucleotides are derivatized in a way that allows their specific and efficient incorporation into the growing strand.

More than 100 different nucleoside- and nucleotide-derivatives are commercially available or can be made using simple techniques (Eaton, Current Opinion in Chemical Biology, 1997, 1: 10-16). Moreover, many nucleotide-derivatives, modified on the bases or the riboses, are incorporated efficiently and specifically by various polymerases, in particular T7 RNA polymerase and Reverse Transcriptase (Figure 9). Nucleotides with additions of up to 300 Da have been incorporated specifically and efficiently (Wiegand et al., Chemistry and Biology, 1997, 4: 675-683; Fenn and Herman, Analytical Chemistry, 1990, 190: 78-83; Tarasow and Eaton, Biopolymers, 1998, 48: 29-37). In addition to the four natural base pairs (AT or AU, TA or UA, CG, GC), at least 8 base pairs are known to hybridise specifically, some of which are incorporated into oligonucleotides by polymerases in a template-dependent manner.

The incorporation of complementing elements may be catalyzed by chemical or biological catalysts. When the building blocks are nucleotides, particularly relevant catalysts are template-dependent DNA- and RNA-polymerases, including reverse transcriptases, and DNA- and RNA- ligases, ribozymes and deoxyribozymes. Specific examples include HIV-1 Reverse Transcriptase, AMV Reverse Transcriptase, T7 RNA polymerase and T7 RNA polymerase mutant Y639F, Sequenase, Taq DNA polymerase, Klenow Fragment (Large fragment of DNA polymerase I), DNA-ligase, T7 DNA polymerase, T4 DNA polymerase, T4 DNA Ligase, E. coli RNA polymerase, rTh DNA polymerase, Vent DNA polymerase, Pfu DNA polymerase, Tte DNA polymerase, ribozymes with ligase or replicase activities such as described in (Johnston et al., Science, May 18, 2001, pp. 1319-1325), and other enzymes that accept nucleotides and/or oligonucleotides as substrates. Mutant or engineered polymerases with improved characteristics, for example broadened nucleotide substrate specificity, and mutants in which the proofreading function has been eliminated (for example by deleting the nuclease activity), are particularly relevant. The polymerases may

use single or double stranded nucleotides as templates, and produce single or double stranded nucleotide products.

Sites of modification that have been shown to be accepted by polymerases include the following non-exhaustive list of examples (See also Figure 9):

Nucleotide	Site of modification
dATP	3-position
dATP	8-position
dATP	2' (deoxyribose moiety)
dTTP	4' (deoxyribose moiety)
dGTP	7-position
dCTP	2' (deoxyribose moiety)
dUTP	2' (deoxyribose)
UTP	5-position
ATP	8-position

Terminal transferase, RNA ligases, Polynucleotide kinases and other template independent enzymes that accept nucleotides and/or oligonucleotides as substrates, including engineered or mutant variants, may be used for some of the applications and method variations described in the present invention.

It may be possible to attach the functional entities at other sites in the nucleotide, without eliminating hybridization or incorporation specificity. Particularly when employing complementing elements that are di-, tri- or polynucleotides, it may be possible to attach functional entities at these alternative sites without inhibiting specific incorporation.

Cleavable and non-cleavable linkers

A selection of cleavable linkers and protection groups, as well as the agents that cleave them, are illustrated in (Figure 10).

It is preferred that linkers do not react with other linkers, complementing elements or functional entities, in the same monomer or in another monomer. Also, in some of the schemes proposed herein, it is desirable that the linker is not cleaved by the conditions of polymerization. Finally, it is preferred that the conditions of linker cleavage does not affect the integrity of the template, complementing template or functional entities.

Linkers can be cleaved in any number of ways when subjected to predetermined conditions. Linkers may e.g. be cleaved with acid, base, photolysis, increased temperature, added agents, enzymes, ribozymes or other catalysts. Examples of cleavable linkers and their respective protection groups are shown in (Figure 10), along with the conditions for linker cleavage, and the cleavage products.

To maintain a physical link between the template and the templated molecule, at least one non-cleavable linker is needed. This non-cleavable linker is preferably flexible, enabling it to expose the templated molecule in an optimal way.

Polymerization

Reactions that lead to polymer formation are termed polymerization reactions. The major reaction-classes are anionic polymerizations, cationic polymerizations, radical polymerizations, and pericyclic polymerizations.

Although polymerisation reactions in solution is achievable by state of the art methods, polymerisation of functional entities linked to an array as described herein does not constitute standard type reactions. Only a few polymerisation reactions have so far been performed in an array format, and not in connection with the methods of the present invention. Consequently, it will be a matter of molecular design of the functional entities and their linkers and attachment points on the complementing elements (e.g. attachment to the base, ribose or phosphate of a nucleotide), as well as

a matter of optimising the polymerisation conditions, in order to preferably reduce minimize or even eliminate any undesirable reactions taking place in solution while increasing or maximizing a correct template-directed polymerisation on the array.

The present invention in one embodiment employs polymerization reactions which are in principle known from the state of the art in the sense that they are routinely used in solution synthesis schemes. However, in the present invention, the reactants (reactive groups) are held in close proximity by their attachment to elements of a complementing template. This increases the local concentration significantly. Typical synthesis schemes in solution use 1 μM – 1 mM concentrations of the reactants. When arrayed as disclosed herein, the local concentration will typically be from a thousand-fold to a million-fold higher. As a result, the reactions can in principle be much more efficient. However, the reactions are preferably designed in such a way that the occurrence of undesirable side-reactions are avoided. The molecular design and the polymerization conditions according to the invention reflect this fact and can be further optimised by the skilled person searching for the polymerization conditions and molecular design that maximizes the relative template directed polymerization polymerization in solution.

Depending on the type of initiator and reactive groups, the polymerization may be initiated and/or catalyzed by changes in pH and/or temperature, addition of reactants or catalysts, enzymes or ribozymes, or light, UV or other electromagnetic radiation, etc. Particularly relevant enzymes include proteases, protein ligase (e.g., subtiligase), UDP-glycogen synthetases, CGTases and polyketide synthases. In cases where the conditions and molecular designs have been finely adjusted, so as to allow efficient polymerization of the reactants when arrayed on the complementing template, but insignificant reaction in solution, the polymerization need not be initiated. The increased local concentration in the array simply drives the polymerization.

In the case where incorporation of monomer building blocks are incorporated by an enzyme, one might fuse this enzyme with one of the enzymes mentioned above (e.g., the UDP-glykogen synthetase). This would allow the fusion-protein to first incorporate a monomer through reaction of its reactive groups type I, and right thereafter (as the now-incorporated monomer emerges from the active site of the

enzyme), the other half of the fusion-protein (e.g., the UDP-glykogen synthetase) would link the functional entity of that monomer to the functional entity of the previous monomer in the complementing template.

The functional groups (or backbone structures) may have to be protected, in order to not react with the reactive groups or other components of the system during incorporation, polymerization and activation. This may be achieved using standard protection groups, some of which are mentioned in (Figure 10).

The polymerization reactions described herein below are divided into two major groups, dependent on whether the funtional entity is held in a fixed oritentation relative to the complementing template.

Group 1: The functional entities can rotate relative to the complementing elements (and can therefore rotate relative to the complementing template).

Direct linkage of reactive groups: The reactive group type II of one monomer react directly with the reactive group type II of another monomer.

a). In one example, the functional entity carries two reactive groups X1 and X2 of the same kind. "Same kind" in this respect means that a given X1 can react with both an identical X1 and a non-identical X2. In (Figure 11) X1 and X2 are identical, wherefore they are both symbolized with an X. X may react with another X to form XX (Figure 11). As an example, X might be a thiol (-SH) and the resulting product a disulfide (-SS-). As another example, X could be a coumarin moiety which upon photo-induction reacts with a coumarin moiety of a neighbouring monomer (Figure 11, example 1).

In most cases, the reaction of X with X results in the loss of an atom or a molecular moiety; in the case of the thiol, for example, two protons are lost upon disulfide formation. The fact that XX (the result of the reaction between two reactive groups type II) does not contain all the components of X plus X, is indicated in (Figure 5, A) where in fact both types of reactive groups (both type I and II) upon reaction forms a

molecular entity that is slightly different from the reactive groups (symbolized by overlapping circles in the figure).

b). The two reactive groups type II may be of a different kind. "Different kind" here means that they react with different types of molecules. For example, X and Y might be nucleophiles and electrophiles, respectively. X and Y react to form XY (Figure 12). For longer templated molecules, free rotation of the functional entities relative to the complementing template represents a potential problem, if the functional entities do not react until many monomers have been incorporated. In this case, cluster formation (Figure 13) may result, which decreases the amount of full-length, templated polymers. The problem is, however, only significant for longer polymers; from experience with biological display of α-peptides, such as phage-display and polysome-display, it is known that display efficiencies as low as 1 % is enough to isolate peptides with high binding affinity for a given target.

In certain cases the incorporated monomers react right after their incorporation into the complementing template (at which time the next monomer in the complementing template has not been incorporated yet). Therefore, the last incorporated monomer will react with the second-last incorporated monomer, which is already part of the complementing template. As a result, cluster formation will not be a significant problem in this case.

X and Y might be an amine and a carboxylic acid. In the presence of carbodiimide, X and Y will react to form an amide XY. Other examples of X and Y combinations, and the resulting products, are shown in Figure 25.

Another version of this type of polymerization involves the simultaneous polymerization and activation of the polymer (Figure 14). The monomers do not contain a separate linker moiety; rather, the polymerization reaction leads to activation (release of the funtional entity from the complementing template). In this scheme, each monomer is incorporated and reacts with the previously incorporated monomer, leading to the previously incorporated monomers release from the complementing template, before the next monomer is incorporated. (Figure 14, example 1) shows the use of this principle for the formation of polyamides, in this case β-peptides. The

method may obviously be used for other peptides also, as well as any kind of polyamides.

By appropriate design of the monomers, one may generate other types of polymer bonds by nucleophilic substitution reactions, including amide, ester, carbamate, carbonate, phosphonate, phosphodiester, sulfonamide, urea, carbopeptide, glycopeptide, saccharide, hydrazide, disulfide and peptoid bonds.

In (Figure 14, example 2) the same principle is applied to a different type of reaction, a "rolling circle polymerization reaction". An alkyl sulfonate is here used as an efficient leaving group, to drive the formation of a secondary amine. The result is a functionalized polyamine, attached at one end to the template that directed its synthesis. In an analogous way, one may generate polyether and poly-thioether using similar molecular designs. Polymers that can be generated by the use of the principles described in (Figure 14 and 14, example 1 and 2) include oligodeoxynucleotides, oligoribonucleotides, chimeric oligonucleotides, oligonucleotide analogs (e.g., PNA, LNA), peptoids, polypeptides and β-peptides.

<u>"Fill-in" polymerization: An additional molecule mediates linkage between reactive groups type II from neighbouring monomers.</u>

a). The functional entitiy carry one or two reactive groups X1 and X2 of the same kind, where X1 cannot react with another X1 or X2. For example, X1 and X2 could be a primary and secondary amine, respectively. In order to polymerize, a compound of the kind Y1-linker-Y2 is added, where Y1 and Y2 are of the same kind. Y can react with X, but is sterically or chemically excluded from reaction with another Y. As a result, a X-Y-Y-X is formed (Figure 15). As an example, X could be an amine, and Y a activated ester. Upon reaction, this would form an ester-ester bond (X-Y-Y-X) between two functional entities.

It is preferred that the two X of one monomer does not to any significant extent react with the same Y-linker-Y molecule. This can be prevented e.g. by imposing steric constraints on the molecules, e.g., Ys in the Y-linker-Y molecule are further apart than the Xs in the monomer.

(Figure 15, example 2) provides two examples of "fill-in" polymerization of polyamides. In (Figure 15, example 2, A and B), the reactive groups type II are amines, and the Y-linker-Y molecule is a dicarboxylic acid or an activated di-ester. In either case, the resulting product is a di-amide polymer. Obviously, the kind of X and Y could be switched, so that in the examples X was a carboxylic acid and Y an amine. Other combinations of X and Y, and their resulting bonds, are given in Figure 25, which summarizes some of the kinds of polymers that may be generated by the various polymerization principles described in the present patent..

For certain reactions, the linking molecule need only contain one reactive group X. An example is shown in (Figure 15, example 3A), where the functional entities contain two reactive groups type II (amines), and the added molecule is a phosgen equivalent such as 1,1'-carbonyldiimidazole. The resulting bond is an urea bond. In (Figure 15, example 5) the monomers contain two hydroxyl groups, to which is added an activated phosphodiester or an activated phosphine derivative such as a bisaminophosphine following activation with tetrazole and oxidation with *tert*-butylhydroperoxide. The result is a phosphodiester bond.

The functional entity may in certain cases contain only one reactive group type II. An example is shown in (Figure 15, example 6), where an activated phosphodiester makes up the only reactive group type II of the monomer. Upon reaction with a dihydroxy, a phosphodiesterbackbone is formed.

As yet another example of fill-in polymerization, (Figure 15, example 7) shows the pericyclic reaction of dienes (functional entity) reacting with alkenes (linking molecule), to form a polycyclic compound.

A general consideration when using the fill-in polymerization principle, is the number of stereoisomers templated by the same template. For example, in (Figure 15, example 4, A), the functional entity contains two primary amines. The functional entity is connected to the complementing template through a chiral carbon. The functional entity may rotate freely around the bond that connects this chiral atom with the complementing template. Therefore, the reaction of the amines (X) with the linking molecules (activated carbonyls, (Y)) will result in the formation of 2^n different isomers, where n is the number of residues of the polymer.

The isomers represent a significant increase in diversity. For example, for a 10-meric polymer, the chirality represents a 1024-fold increase in diversity. This may in certain cases be an advantage, for example if the monomer diversity is low, or if the desire is to make short polymers. However, such "scrambling" of the genetic code (i.e., one template encodes different polymer structures) also decreases the stringency of the selection process. Therefore, in certain cases scrambling is not desirable. One may then choose to connect the functional entities to the complementing elements via non-chiral atoms. In (Figure 15, example 4, B) is shown an example of an achiral atom (nitrogen) connecting the functional entity with the complementing template. Scrambling may involve cases where one complementing element specifies different isomers (as described above), and scrambling may also involve cases where a complementing element specifies slightly different or entirely different functional entities.

b). The functional entity carries two different reactive groups of type II, X and S (Figure 16). X does not react with X or S, and vice cersa. Before, during or after incorporation of monomers, molecules of the form T-linker-Y are added. X may react with Y, and S may react with T, leading to formation of X-S-T-Y linkages between the functional entities. It is important to ensure that X and S of one functional entity cannot react with T and Y of one linking molecule. This may be ensured by appropriate design of the structure of the functional entities and linking molecule. (Figure 16, example 1) provides an example of a functional entity with different reactive groups type II, in this case an azide and a hydrazide (X and S), and a linking molecule with different reactive groups, in this case a phosphine and a ketone (T-linker-Y).

For longer templated molecules, free rotation of the functional entities relative to the complementing template represents a potential problem, if the functional entities do not react until many monomers have been incorporated. In this case, cluster formation (Figure 13) may result, which decreases the amount of full-length, templated polymers. The problem is, however, only significant for longer polymers, as explained above. If the linking molecules are present during incorporation of the complementing elements, the incorporated monomers may react with the linking molecules right after their incorporation, or in the case of enzyme-mediated incorporation,

as soon as they emerge from the active site of the enzyme. Cluster formation will not be a significant problem in these cases.

"Zipping" polymerization: The polymerization reaction travels from one end of the template to the other.

In this approach, the polymerization reaction is directional, i.e., the reaction cascade starts at one end of the complementing template, and the reactions migrate to the other end of the complementing template, thereby forming a templated polymer.

a). General principle (Figure 17). After incorporation of some or all of the monomer building blocks, polymerization is initiated from one end of the template, and travels down the template. For example, the initiator may be coupled to the first or last complementing element to be incorporated, or it may be coupled to the primer used in DNA polymerase-mediated incorporation of nucleotide-derivatives. Either way, the initiator will react with the neighbouring monomer's reactive group type II, which induces a change in the functional entity of that monomer, allowing this monomer to react with the next monomer in the chain, and so on. Eventually, all the monomers have reacted, and a polymer has been formed.

It may be desirable to protect the initiator, keeping it from reacting with the neighbouring monomer until incorporation is complete, whereafter the initiator is deprotected. This allows the experimenter to remove all non-incorporated initiators and complementing elements before activating the initiator, which eliminates reaction in solution between the initiator and the complementing elements.

Deprotection of the initiator may be by change in pH or temperature, exposure to electromagnetic radiation, or addition of an agent (that removes a protection group, or introduces an initiator at a specific position, or ligates or coordinates to the naïve initiator, to make it a more potent initiator). The agent could be a chemical catalyst or an enzyme, for example an esterase or peptidase.

b). Zipping by radical polymerization (Figure 17, example 1). The initiator is a alkyl-iodide, and the funtional entities contain a double bond. Upon addition of a radical initiator, for example ammoniumpersulfate, AIBN (azobis-isobutyronitrile) or other

radical chain reaction initiators, a radical chain reaction is initiated, whereby the alkenes react to form an extended, functionalized alkane. Eventually, the polymer has been made, and it is activated (cleaved from the complementing template, except at one point). The radical remaining at the end of the polymer may be quenched by a radical termination reaction.

c). Zipping by cationic polymerization (Figure 17, example 2). The initiator is a Lewis acid. Upon deprotection with acid or other initiation reagent, a cation is generated. The carbocation attacks the double bond of the neighbouring monomer, and as a result a carbocation is generated in this monomer. Eventually, the full-length polymer has been formed, and the polymer is activated.

d). Zipping by nucleophilic (anionic) polymerization (Figure 18, example 3). In this example, the initiator is a protected hydroxyl anion. The functional entity carries a peroxide. Upon deprotection of the initiator the hydroxyl-anion is formed (e.g., by alkaline deprotection). Under basic conditions, the initiator attacks the neighbouring epoxide at the least hindered carbon in the ring. This in turn generates a hydroxyl-anion, which attacks the neighbouring epoxide. Eventually, the full polymer is formed, and the polyether may be activated. In this example, all of the linkers that connect the polyether to the complementing template are cleaved.
This type of polymerization is also an example of ring-opening polymerization.

e). Zipping polymerization by ring opening (Figure 18). The general principle of ring-opening polymerization is shown. The initiator attacks the reactive group X of the neighbouring monomer. X is part of a ring structure, and as a result of the reaction between the initiator and X, the ring opens, whereby the other reactive group of the monomer is activated for attack on the next monomer in the array. Polymerization travels down the strand, and eventually the full-length polymer has been formed.

f). β-peptide formation by ring-opening polymerization of carboxyanhydrides (Figure 18, example 1). The deprotected initiator, a nucleophilic amine, attacks the most electrophilic carbonyl of the N-thiocarboxyanhydride, to form an amide. CSO is released, generating a primary amine, which then attacks the next monomer in the array. Eventually polymerization is complete, and the polymer may be activated,

creating a β-peptide attached to the complementing template or template through its C-terminal end. The principle may be used to form other types of peptides, for example D- and L- form mono- and disubstituted α-peptides, β-peptides, γ-peptides, carbopeptides and peptoids (poly N-substituted glycin), and other types of polyamides. Also, the principle can be employed for the generation of other polymers, such as polyesters, polyureas, and polycarbamates.

g). β-peptide formation by ring-opening polymerization of thiazinanone units (Figure 18, example 2). The deprotected initiator attacks the cyclic thioester, to form an amide. As a result, the ring breaks down to release a free thioketone. This generates an amine, which may now attack the thioester of the next monomer in the array. When polymerization has travelled to the other end of the template, it is activated, generating a β-peptide attached to its template through the C-terminal end.

The principle may be used to form other types of peptides, for example D- and L-form mono- and disubstituted α-peptides, β-peptides, γ-peptides, carbopeptides and peptoids (poly N-substituted glycin), and other types of polyamides. Also, the principle can be employed for the generation of other polymers, such as polyesters, polyureas, and polycarbamates.

h) Zipping polymerization by rearrangement (Figure 19). Upon activation of the initiator, which in this case could be an electrophile, the reactive group type II of the neighbouring monomer attacks the initiator, and as a result, releases the initiator from the complementing element. In the attacking monomer, the reaction of Y with the initiator leads to a rearrangement of the monomer, which results in activation of X, the other reactive group type II of the monomer (for example, the reorganization creates a nucleophile). Then, the next monomer in the array attacks this nucleophile. Eventually, full-length polymer has been formed, attached at one end to the template that directed its synthesis.

i). Zipping and activation in one step (Figure 20). By appropriate design of the functional entities used for ring-opening polymerization, activation may be achieved as a direct result of the polymerization reaction. By simply turning the functional entity upside-down, i.e., attach the portion of the ring that does not get incorporated into the final polymer to the complementing template, saves the experimenter an

activation step (compare Figure 20 and Figure 18). As a specific example, attachment of the 2,2-diphenylthiazinanone ring structure of (Figure 18, example 2) to the complementing elements through one of the phenyl groups would lead to activation as a result of the polymerization reaction.

Group 2: The functional entitiy cannot rotate freely relative to the complementing element

In this embodiment, the X and Y reactive groups type II are held in the desired orientation relative to the complementing template (Figure 21, A). X and Y can therefore react, or react with a linker molecule, without the risk of cluster formation (compare with Figure 13).

The functional entity may be held in the fixed orientation by a double bond, or by bonds to different atoms in the complementing element. (Figure 21, B) provides an example, where the functional entity is covalently coupled to the two bases of a dinucleotide (the complementing element is a dinucleotide, the functional entity contains a dipeptide, and the reactive groups are the amine and the ester moieties, respectively).

Polymers that can be made by this method include all of the polymers mentioned in the non-zipping polymerizations above, for example peptides, amides, esters, carbamates, oximes, phosphodiesters, secondary amines, ethers, etc.

Figures 22-25 gives an overview in the form of a non-exhaustive and non-limiting list of polymers capable of being templated using the above-mentioned principles. Figure 22 lists selected examples of polymers. Figure 23 provides selected examples of precursors (functional entities) that may be used for the templated synthesis of various polymers. Figure 24 lists selected functional groups, or molecular entities, that may be used. Figure 25 provide selected examples of reactive groups that may be used to generate specific templated molecules, and also refers to relevant figures describing the principles that may be used in the templating process.

Linker cleavage

Activation (cleavage of some or all of the linkers connecting the complementing elements and the functional entity) may be done by changes in pH and/or temperature, addition of reactants or catalysts, enzymes or ribozymes, or light, UV or other electromagnetic radiation, etc. Particularly relevant enzymes include proteases, esterases and nucleases. A list of cleavable linkers and the conditions for cleavage is shown in (Figure 10).

Other cleavable linkers include the 4-hydroxymethyl phenoxyacetic acid moiety, which is cleaved by acid, the 2-[(tert-butyldiphenylsiloxy)methyl]benzoic acid moiety which is celavable with fluoride, and the phosphate of a 2-hydroxymethyl benzoic acid moiety which provides a linker cleavable by the combination of alkaline phosphatase treatment followed by treatment with mild alkaline treatment.

In most cases, it is desirable to have at least two different types of linkers connecting the complementing elements with the funtional entities. This way, it is possible to selectively cleave all but one of the linkers between the complementing template and the functional entities, thereby obtaining a polymer physically linked through just one linker to the template that templated its synthesis,. This intact linker should affect the activities of the attached polymer as little as possible, but other than that, the nature of the linker is not considered an essential feature of this invention. The size of the linker in terms of the length between the template and the templated polymer can vary widely, but for the purposes of the invention, preferably the length is in the range from the length of just one bond, to a chain length of about 20 atoms.

Selection and screening of templated molecules

Selection or screening of the templated molecules with desired activities (for example binding to particular target, catalytic activity, or a particular effect in an activity assay) may be performed according to any standard protocol. For example, affinity selections may be performed according to the principles used for phage displayed, polysome-displayed or mRNA-protein fusion displayed peptides. Selection for catalytic activity may be performed by affinity selections on transition-state analog affinity columns (Baca et al. , Proc. Natl. Acad. Sci USA. 1997; 94(19):10063-8), or by

function-based selection schemes (Pedersen et al., Proc. Natl. Acad. Sci. USA. 1998, 95(18):10523-8). Screening for a desired characteristic may be performed according to standard microtiter plate-based assays, or by FACS-sorting assays.

Amplification of templates capable of templating the synthesis of templated molecules

In one aspect the present invention relates to methods for amplifying templated molecules that may or may not be bound to a target. The choice of amplification method depends on the choice of coding or complementing elements. Natural oligonucleotides can be amplified by any state of the art method. These methods include, but is not limited to the polymerase chain reaction (PCR); as wells as e.g. nucleic acid sequence-based amplification (e.g. Compton, Nature 350, 91-92 (1991)), amplified anti-sense RNA (e.g. van Gelder et al., PNAS 85: 77652-77656 (1988)); self-sustained sequence replication system (e.g. Gnatelli et al., PNAS 87: 1874-1878 (1990)); polymerase independent amplification as described in e.g. Schmidt et al., NAR 25: 4797-4802 (1997), as well as in vivo amplification of plasmids carrying cloned DNA fragments. Ligase-mediated amplification methods may also be used, e.g., LCR (Ligase Chain Reaction).

For non-natural nucleotides the choices of efficient amplification procedures are fewer. As non-natural nucleotides per definition can be incorporated by certain enzymes including polymerases, it will be possible to perform manual polymerase chain reaction by adding the polymerase during each extension cycle.

For oligonucleotides containing nucleotide analogs, fewer methods for amplification exist. One may use non-enzyme mediated amplification schemes (Schmidt et al., NAR 25: 4797-4802 (1997)). For backbone-modified oligonucleotide analogs such as PNA and LNA, this amplification method may be used. Before or during amplification the templates or complementing templates may be mutagenized or recombined in order to create a larger diversity for the next round of selection or screening.

Characterization of polymers isolated by the selections or screening assays.

After the final round of selection, it is often desirable to sequence individual templates, in order to determine the sequence of individual templated polymers. If the template contains natural nucleotides, it is a standard routine to optionally PCR amplify the isolated templates (if the template is an RNA molecule, it is necessary to use reverse transcriptase to produce cDNA prior to the PCR-amplification), and then clone the DNA fragments into for example plasmids, transform these and then sequence individual plasmid-clones containing one or multiple tandem DNA sequences. In this case, it is practical to design a restriction site in both of the flanking sequences to the central random or partly random sequence of the template (i.e., in the primer binding sites). This will allow easy cloning of the isolated nucleotides. Sequencing can be done by the standard dideoxy chain termination method, or by more classical means such as Maxam-Gilbert sequencing.

If the template contains non-natural nucleotides, it is not feasible to clone individual sequences by transfer through a microbial host. However, using bead populations where each bead carries one oligonucleotide sequence, it is possible to clone in vitro, whereafter all the nucleotides attached to a specific bead may be optionally amplified and then sequenced (Brenner et al., 2000, Proc. Natl. Acad. Sci. USA 97, 1665-1670). Alternatively, one may dilute the population of isolates adequately, and then aliquot into microtiter plates so that the wells on average contain for example 0.1 templates. By amplifying the single templates by for example PCR, it will now be possible to sequence using standard methods. Of course, this requires that the non-natural nucleotides are substrates for the thermostable polymerase used in the PCR.

If alternative methods are used that require shorter oligonucleotides it may be desirable to design the starting template so as to contain restriction sites on either side of the encoding/templating region of the template. Thereby, after the final selection round, the templates can be restricted, to obtain a short oligonucleotide encoding the templated polymer, and then these short oligos can be applied to various analytical procedures.

It is also possible to sequence the isolates by the use of a DNA array of oligos with random but predetermined sequences.

It may also be desirable to sequence the population of isolates as a pool, for example if the sequences are expected to be in register, for example because the initial library consisted of a degenerate sequence based on a polymer sequence with a known (relatively high) desired activity. Therefore, it is then expected that all the isolates have sequences similar to the initial sequence of the templates before selection. Wherefore the population of isolates can be sequenced as a whole, to obtain a consensus sequence for the population as a whole.

Templates

In one embodiment, the templated molecule is linked by means of a single linker to the complementing template or template that templated the synthesis of the templated molecule. In another embodiment, the method for templating a templated molecule comprises the further step of releasing the template or complementing template that templated the templated molecule, and obtaining a templated molecule that is not linked to the complementing template or template that templated the synthesis of the templated molecule.

The template preferably comprises n coding elements in a linear sequence. The template comprising n coding elements can also be branched. n preferably has a value of from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for

example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

In some embodiments of the invention it is preferred that the template is attached to a solid or semi-solid support.

The template in one embodiment preferably comprises or essentially consists of nucleotides selected from the group consisting of deoxyribonucleic acids (DNA), ribonucleic acids (RNA), peptide nucleic acids (PNA), locked nucleic acids (LNA), and morpholinos sequences, including any analog or derivative thereof.

In another embodiment, the template of coding elements preferably comprises or essentially consists of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof, and the complementing element preferably comprises or essentially consists of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

It is preferred in various embodiments of the invention that the template can be characterised by any one or more of the following features: i) That the template is amplifyable, ii) that the template comprises a single strand of coding elements, preferably a single strand of coding elements capable of forming a double helix by hybridization to a complementing template comprising a single strand of complementing elements, and iii) that the template comprises a priming site.

Coding elements

Each coding element is preferably linked to a neighbouring coding element by a covalent chemical bond. Each coding element can also be linked to each neighbouring coding element by a covalent chemical bond. The covalent chemical bond is preferably selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds. More preferably, the covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds and phosphorothioate bonds.

In preferred embodiments, at least one coding element is attached to a solid or semi-solid support.

The coding elements are selected in one embodiment of the inveniton from the group consisting of nucleotides, including any analog or derivative thereof, amino

acids, antibodies, and antigens, and preferably from the group consisting of nucleotides, nucleotide derivatives, and nucleotide analogs, including any combination thereof. In another embodiment, the coding elements are selected from the group consisting of nucleotides, including nucleotides such as deoxyribonucleic acids comprising a base selected from adenine (A), thymine (T), guanine (G), and cytosine (C), and ribonucleic acids comprising a base selected from adenine (A), uracil (U), guanine (G), and cytosine (C). Also in this case can each nucleotide be linked to a neighbouring nucleotide by means of a covalent bond, or linked to each neighbouring nucleotide by means of a covalent bond. The covalent bond is preferably a phosphodiester bond or a phosphorothioate bond.

In other embodiments, the coding elements are natural and non-natural nucleotides selected from the group consisting of deoxyribonucleic acids and ribonucleic acids.

Coding Elements and Corresponding Complementing Elements

When the coding elements are preferably selected from the group consisting of nucleotides, nucleotide derivatives and nucleotide analogs in which one or more of the base moiety and/or the phosphate moiety and/or the ribose or deoxyribose moiety have been substituted by an alternative molecular entity, corresponding complementing elements are capable of interacting with said coding elements and preferably comprise or essentially consist of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof. Each nucleotide is linked to a neighbouring nucleotide by a covalent chemical bond, or linked to each neighbouring nucleotide by a covalent chemical bond. The covalent chemical bond is preferably selected from the group of covalent bonds consisting of phosphodiester bonds and peptide bonds.

Coding Element Subunits

Coding elements in one embodiment preferably comprise or essentially consist of from 1 to 100 subunits, such as from 1 to 80 subunits, for example from 1 to 60 subunits, such as from 1 to 40 subunits, for example from 1 to 20 subunits, such as from 1 to 18 subunits, for example from 1 to 16 subunits, such as from 1 to 14 subunits, for example from 1 to 12 subunits, such as from 1 to 10 subunits, for

example from 1 to 9 subunits, such as from 1 to 8 subunits, for example from 1 to 7 subunits, such as from 1 to 6 subunits, for example from 1 to 5 subunits, such as from 1 to 4 subunits, for example from 1 to 3 subunits, such as from 1 to 2 subunits, for example 1 subunit, such as from 2 to 100 subunits, such as from 2 to 80 subunits, for example from 2 to 60 subunits, such as from 2 to 40 subunits, for example from 2 to 20 subunits, such as from 2 to 18 subunits, for example from 2 to 16 subunits, such as from 2 to 14 subunits, for example from 2 to 12 subunits, such as from 2 to 10 subunits, for example from 2 to 9 subunits, such as from 2 to 8 subunits, for example from 2 to 7 subunits, such as from 2 to 6 subunits, for example from 2 to 5 subunits, such as from 2 to 4 subunits, for example from 2 to 3 subunits, such as 2 subunits, such as from 3 to 100 subunits, such as from 3 to 80 subunits, for example from 3 to 60 subunits, such as from 3 to 40 subunits, for example from 3 to 20 subunits, such as from 3 to 18 subunits, for example from 3 to 16 subunits, such as from 3 to 14 subunits, for example from 3 to 12 subunits, such as from 3 to 10 subunits, for example from 3 to 9 subunits, such as from 3 to 8 subunits, for example from 3 to 7 subunits, such as from 3 to 6 subunits, for example from 3 to 5 subunits, such as from 3 to 4 subunits, for example 3 subunits, for example from 4 to 100 subunits, such as from 4 to 80 subunits, for example from 4 to 60 subunits, such as from 4 to 40 subunits, for example from 4 to 20 subunits, such as from 4 to 18 subunits, for example from 4 to 16 subunits, such as from 4 to 14 subunits, for example from 4 to 12 subunits, such as from 4 to 10 subunits, for example from 4 to 9 subunits, such as from 4 to 8 subunits, for example from 4 to 7 subunits, such as from 4 to 6 subunits, for example from 4 to 5 subunits, for example 4 subunits, such as from 5 to 100 subunits, such as from 5 to 80 subunits, for example from 5 to 60 subunits, such as from 5 to 40 subunits, for example from 5 to 20 subunits, such as from 5 to 18 subunits, for example from 5 to 16 subunits, such as from 5 to 14 subunits, for example from 5 to 12 subunits, such as from 5 to 10 subunits, for example from 5 to 9 subunits, such as from 5 to 8 subunits, for example from 5 to 7 subunits, such as from 5 to 6 subunits, such as 5 subunits, for example from 6 to 100 subunits, such as from 6 to 80 subunits, for example from 6 to 60 subunits, such as from 6 to 40 subunits, for example from 6 to 20 subunits, such as from 6 to 18 subunits, for example from 6 to 16 subunits, such as from 6 to 14 subunits, for example from 6 to 12 subunits, such as from 6 to 10 subunits, for example from 6 to 9 subunits, such as from 6 to 8 subunits, for example from 6 to 7 subunits, such as 6 subunits, such as from 7 to 100 subunits, such as from 7 to 80

subunits, for example from 7 to 60 subunits, such as from 7 to 40 subunits, for example from 7 to 20 subunits, such as from 7 to 18 subunits, for example from 7 to 16 subunits, such as from 7 to 14 subunits, for example from 7 to 12 subunits, such as from 7 to 10 subunits, for example from 7 to 9 subunits, such as from 7 to 8 subunits, such as 7 subunits, for example from 8 to 100 subunits, such as from 8 to 80 subunits, for example from 8 to 60 subunits, such as from 8 to 40 subunits, for example from 8 to 20 subunits, such as from 8 to 18 subunits, for example from 8 to 16 subunits, such as from 8 to 14 subunits, for example from 8 to 12 subunits, such as from 8 to 10 subunits, for example from 8 to 9 subunits, for example 8 subunits, such as from 9 to 100 subunits, such as from 9 to 80 subunits, for example from 9 to 60 subunits, such as from 9 to 40 subunits, for example from 9 to 20 subunits, such as from 9 to 18 subunits, for example from 9 to 16 subunits, such as from 9 to 14 subunits, for example from 9 to 12 subunits, such as from 9 to 10 subunits, such as 9 subunits, for example from 10 to 100 subunits, such as from 10 to 80 subunits, for example from 10 to 60 subunits, such as from 10 to 40 subunits, for example from 10 to 20 subunits, such as from 10 to 18 subunits, for example from 10 to 16 subunits, such as from 10 to 14 subunits, for example from 10 to 12 subunits, such as 10 subunits, such as from 11 to 100 subunits, such as from 11 to 80 subunits, for example from 11 to 60 subunits, such as from 11 to 40 subunits, for example from 11 to 20 subunits, such as from 11 to 18 subunits, for example from 11 to 16 subunits, such as from 11 to 14 subunits, for example from 11 to 12 subunits, such as from 12 to 100 subunits, such as from 12 to 80 subunits, for example from 12 to 60 subunits, such as from 12 to 40 subunits, for example from 12 to 20 subunits, such as from 12 to 18 subunits, for example from 12 to 16 subunits, such as from 12 to 14 subunits, for example from 13 to 100 subunits, such as from 13 to 80 subunits, for example from 13 to 60 subunits, such as from 13 to 40 subunits, for example from 13 to 20 subunits, such as from 13 to 18 subunits, for example from 13 to 16 subunits, such as from 13 to 14 subunits, for example from 14 to 100 subunits, such as from 14 to 80 subunits, for example from 14 to 60 subunits, such as from 14 to 40 subunits, for example from 14 to 20 subunits, such as from 14 to 18 subunits, for example from 14 to 16 subunits, such as from 15 to 100 subunits, such as from 15 to 80 subunits, for example from 15 to 60 subunits, such as from 15 to 40 subunits, for example from 15 to 20 subunits, such as from 15 to 18 subunits, for example from 15 to 16 subunits, such as from 16 to 100 subunits, such as from 16 to 80 subunits, for example from 16 to 60 subunits, such as from 16 to 40 subunits, for

example from 16 to 20 subunits, such as from 16 to 18 subunits, for example from 17 to 100 subunits, such as from 17 to 80 subunits, for example from 17 to 60 subunits, such as from 17 to 40 subunits, for example from 17 to 20 subunits, such as from 17 to 18 subunits, for example from 18 to 100 subunits, such as from 18 to 80 subunits, for example from 18 to 60 subunits, such as from 18 to 40 subunits, for example from 18 to 20 subunits, such as from 19 to 100 subunits, such as from 19 to 80 subunits, for example from 19 to 60 subunits, such as from 19 to 40 subunits, for example from 19 to 30 subunits, such as from 19 to 25 subunits, for example from 20 to 100 subunits, such as from 20 to 80 subunits, for example from 20 to 60 subunits, such as from 20 to 40 subunits, for example from 20 to 30 subunits, such as from 20 to 25 subunits.

In preferred embodiments, each coding element subunit comprises or essentially consists of a nucleotide, or a nucleotide analog. The nucleotide can be a deoxyribonucleic acid comprising a base selected from adenine (A), thymine (T), guanine (G), and cytosine (C), or it can be a ribonucleic acid comprising a base selected from adenine (A), uracil (U), guanine (G), and cytosine (C). Each nucleotide is linked to a neighbouring nucleotide, or nucleotide analog, by means of a covalent bond, or linked to each neighbouring nucleotide, or nucleotide analog, by means of a covalent bond, including covalent bonds selected from the group consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

In one embodiment it is preferred that at least some of said nucleotides are selected from the group consisting of nucleotide derivatives, including deoxyribonucleic acid derivatives and ribonucleic acid derivatives.

Coding Element Subunits and Corresponding Complementing Element Subunits

The coding element subunits are preferably selected from the group consisting of nucleotides, nucleotide derivatives and nucleotide analogs in which one or more of a base moiety and/or a phosphate moiety and/or a ribose moiety and/or a deoxyribose moiety have been substituted by an alternative molecular entity, and the corresponding complementing element subunits capable of interacting with said

coding element subunits comprise or essentially consist of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

Each nucleotide derivative can be linked to a neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond, or each nucleotide derivative can be linked to each neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond. The covalent chemical bond is preferably selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

Complementing Elements

The complementing template in one embodiment preferably comprises n complementing elements in a linear sequence or a branched sequence. n preferably has a value of from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, such as 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, such as 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, such as 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, for example 8, such as 9, for

example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

In some embodiments, the complementing template is attached to a solid or semi-solid support.

The complementing template in one embodiment comprises or essentially consists of nucleotides selected from the group consisting of deoxyribonucleic acids (DNA), ribonucleic acids (RNA), peptide nucleic acids (PNA), locked nucleic acids (LNA), and morpholinos sequences, including any analog or derivative thereof.

In other embodiments, there is provided a complementing template comprising or essentially consisting of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof, wherein the corresponding coding elements of the template comprise or essentially consist of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

The complementing template is preferably amplifyable and/or comprises a single strand of complementing elements and/or comprises a single strand of complementing elements capable of forming a double helix by hybridization to a template comprising a single strand of coding elements, and/or comprises a priming site.

Each complementing element is preferably linked to a neighbouring complementing element by a covalent chemical bond, or linked to each complementing element is linked to each neighbouring complementing element by a covalent chemical bond. The covalent chemical bond is in one embodiment selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds. In other embodiments, the group of covalent bonds consist of phosphodiester bonds and phosphorothioate bonds.

The at least one complementing element can be attached to a solid or semi-solid support.

The complementing elements can be selected from the group consisting of nucleotides, including any analog or derivative thereof, amino acids, antibodies, and antigens, and preferably from the group consisting of nucleotides, nucleotide derivatives, and nucleotide analogs, including any combination thereof. In one embodiment, it is preferred that the complementing elements are selected from the group consisting of nucleotides, including deoxyribonucleic acids comprising a base selected from adenine (A), thymine (T), guanine (G), and cytosine (C), and ribonucleic acids comprising a base selected from adenine (A), uracil (U), guanine (G), and cytosine (C).

Each nucleotide can be linked to a neighbouring nucleotide, or nucleotide analog, by means of a covalent bond, including, or each nucleotide can be linked to each neighbouring nucleotide, or nucleotide analog, by means of a covalent bond. The covalent bond can be a phosphodiester bond or a phosphorothioate bond.

In another embodiment, the complementing elements are natural or non-natural nucleotides selected from the group consisting of deoxyribonucleic acids and ribonucleic acids.

Complementing Elements and Corresponding Coding Elements

When the complementing elements are selected from the group consisting of nucleotides, nucleotide derivatives and nucleotide analogs in which one or more of a base moiety and/or a phosphate moiety and/or a ribose and/or a deoxyribose moiety has been substituted by an alternative molecular entity, the coding elements capable of interacting with said complementing elements comprise or essentially consist of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

Each nucleotide can be linked to a neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond, or linked to each neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond. The covalent chemical bond is preferably selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

The complementing elements are in one embodiment selected from nucleotides, and the complementing elements can in one preferred embodiment be linked enzymatically by using an enzyme selected from the group consisting of template-dependent DNA- and RNA-polymerases, including reverse transcriptases, DNA-ligases and RNA-ligases, ribozymes and deoxyribozymes, including HIV-1 Reverse Transcriptase, AMV Reverse Transcriptase, T7 RNA polymerase, T7 RNA polymerase mutant Y639F, Sequenase, Taq DNA polymerase, Klenow Fragment (Large fragment of DNA polymerase I), DNA-ligase, T7 DNA polymerase, T4 DNA polymerase, T4 DNA Ligase, E. coli RNA polymerase, rTh DNA polymerase, Vent

DNA polymerase, Pfu DNA polymerase, Tte DNA polymerase, and ribozymes with ligase or replicase activities.

More preferably, the enzyme is selected from the group consisting of HIV-1 Reverse Transcriptase, AMV Reverse Transcriptase, T7 RNA polymerase, T7 RNA polymerase mutant Y639F, Sequenase, Taq DNA polymerase, Klenow Fragment (Large fragment of DNA polymerase I), DNA-ligase, T7 DNA polymerase, T4 DNA polymerase, and T4 DNA Ligase. The nucleotides preferably form a template or complementing template upon incorporation.

In another embodiment, the complementing elements can be selected from nucleotides, and linked by using a chemical agent, pH change, light, a catalyst, radiation, such as electromagnetic radiation, or by spontaneous coupling when being brought into reactive contact with each other.

Complementing Element Subunits

The complementing element preferably comprises or essentially consists of from 1 to 100 subunits, such as from 1 to 80 subunits, for example from 1 to 60 subunits, such as from 1 to 40 subunits, for example from 1 to 20 subunits, such as from 1 to 18 subunits, for example from 1 to 16 subunits, such as from 1 to 14 subunits, for example from 1 to 12 subunits, such as from 1 to 10 subunits, for example from 1 to 9 subunits, such as from 1 to 8 subunits, for example from 1 to 7 subunits, such as from 1 to 6 subunits, for example from 1 to 5 subunits, such as from 1 to 4 subunits, for example from 1 to 3 subunits, such as from 1 to 2 subunits, for example 1 subunit, such as from 2 to 100 subunits, such as from 2 to 80 subunits, for example from 2 to 60 subunits, such as from 2 to 40 subunits, for example from 2 to 20 subunits, such as from 2 to 18 subunits, for example from 2 to 16 subunits, such as from 2 to 14 subunits, for example from 2 to 12 subunits, such as from 2 to 10 subunits, for example from 2 to 9 subunits, such as from 2 to 8 subunits, for example from 2 to 7 subunits, such as from 2 to 6 subunits, for example from 2 to 5 subunits, such as from 2 to 4 subunits, for example from 2 to 3 subunits, such as 2 subunits, such as from 3 to 100 subunits, such as from 3 to 80 subunits, for example from 3 to 60 subunits, such as from 3 to 40 subunits, for example from 3 to 20 subunits, such as from 3 to 18 subunits, for example from 3 to 16 subunits, such as

from 3 to 14 subunits, for example from 3 to 12 subunits, such as from 3 to 10 subunits, for example from 3 to 9 subunits, such as from 3 to 8 subunits, for example from 3 to 7 subunits, such as from 3 to 6 subunits, for example from 3 to 5 subunits, such as from 3 to 4 subunits, for example 3 subunits, for example from 4 to 100 subunits, such as from 4 to 80 subunits, for example from 4 to 60 subunits, such as from 4 to 40 subunits, for example from 4 to 20 subunits, such as from 4 to 18 subunits, for example from 4 to 16 subunits, such as from 4 to 14 subunits, for example from 4 to 12 subunits, such as from 4 to 10 subunits, for example from 4 to 9 subunits, such as from 4 to 8 subunits, for example from 4 to 7 subunits, such as from 4 to 6 subunits, for example from 4 to 5 subunits, for example 4 subunits, such as from 5 to 100 subunits, such as from 5 to 80 subunits, for example from 5 to 60 subunits, such as from 5 to 40 subunits, for example from 5 to 20 subunits, such as from 5 to 18 subunits, for example from 5 to 16 subunits, such as from 5 to 14 subunits, for example from 5 to 12 subunits, such as from 5 to 10 subunits, for example from 5 to 9 subunits, such as from 5 to 8 subunits, for example from 5 to 7 subunits, such as from 5 to 6 subunits, such as 5 subunits, for example from 6 to 100 subunits, such as from 6 to 80 subunits, for example from 6 to 60 subunits, such as from 6 to 40 subunits, for example from 6 to 20 subunits, such as from 6 to 18 subunits, for example from 6 to 16 subunits, such as from 6 to 14 subunits, for example from 6 to 12 subunits, such as from 6 to 10 subunits, for example from 6 to 9 subunits, such as from 6 to 8 subunits, for example from 6 to 7 subunits, such as 6 subunits, such as from 7 to 100 subunits, such as from 7 to 80 subunits, for example from 7 to 60 subunits, such as from 7 to 40 subunits, for example from 7 to 20 subunits, such as from 7 to 18 subunits, for example from 7 to 16 subunits, such as from 7 to 14 subunits, for example from 7 to 12 subunits, such as from 7 to 10 subunits, for example from 7 to 9 subunits, such as from 7 to 8 subunits, such as 7 subunits, for example from 8 to 100 subunits, such as from 8 to 80 subunits, for example from 8 to 60 subunits, such as from 8 to 40 subunits, for example from 8 to 20 subunits, such as from 8 to 18 subunits, for example from 8 to 16 subunits, such as from 8 to 14 subunits, for example from 8 to 12 subunits, such as from 8 to 10 subunits, for example from 8 to 9 subunits, for example 8 subunits, such as from 9 to 100 subunits, such as from 9 to 80 subunits, for example from 9 to 60 subunits, such as from 9 to 40 subunits, for example from 9 to 20 subunits, such as from 9 to 18 subunits, for example from 9 to 16 subunits, such as from 9 to 14 subunits, for example from 9 to 12 subunits, such as from 9 to 10 subunits, such as 9 subunits,

for example from 10 to 100 subunits, such as from 10 to 80 subunits, for example from 10 to 60 subunits, such as from 10 to 40 subunits, for example from 10 to 20 subunits, such as from 10 to 18 subunits, for example from 10 to 16 subunits, such as from 10 to 14 subunits, for example from 10 to 12 subunits, such as 10 subunits, such as from 11 to 100 subunits, such as from 11 to 80 subunits, for example from 11 to 60 subunits, such as from 11 to 40 subunits, for example from 11 to 20 subunits, such as from 11 to 18 subunits, for example from 11 to 16 subunits, such as from 11 to 14 subunits, for example from 11 to 12 subunits, such as from 12 to 100 subunits, such as from 12 to 80 subunits, for example from 12 to 60 subunits, such as from 12 to 40 subunits, for example from 12 to 20 subunits, such as from 12 to 18 subunits, for example from 12 to 16 subunits, such as from 12 to 14 subunits, for example from 13 to 100 subunits, such as from 13 to 80 subunits, for example from 13 to 60 subunits, such as from 13 to 40 subunits, for example from 13 to 20 subunits, such as from 13 to 18 subunits, for example from 13 to 16 subunits, such as from 13 to 14 subunits, for example from 14 to 100 subunits, such as from 14 to 80 subunits, for example from 14 to 60 subunits, such as from 14 to 40 subunits, for example from 14 to 20 subunits, such as from 14 to 18 subunits, for example from 14 to 16 subunits, such as from 15 to 100 subunits, such as from 15 to 80 subunits, for example from 15 to 60 subunits, such as from 15 to 40 subunits, for example from 15 to 20 subunits, such as from 15 to 18 subunits, for example from 15 to 16 subunits, such as from 16 to 100 subunits, such as from 16 to 80 subunits, for example from 16 to 60 subunits, such as from 16 to 40 subunits, for example from 16 to 20 subunits, such as from 16 to 18 subunits, for example from 17 to 100 subunits, such as from 17 to 80 subunits, for example from 17 to 60 subunits, such as from 17 to 40 subunits, for example from 17 to 20 subunits, such as from 17 to 18 subunits, for example from 18 to 100 subunits, such as from 18 to 80 subunits, for example from 18 to 60 subunits, such as from 18 to 40 subunits, for example from 18 to 20 subunits, such as from 19 to 100 subunits, such as from 19 to 80 subunits, for example from 19 to 60 subunits, such as from 19 to 40 subunits, for example from 19 to 30 subunits, such as from 19 to 25 subunits, for example from 20 to 100 subunits, such as from 20 to 80 subunits, for example from 20 to 60 subunits, such as from 20 to 40 subunits, for example from 20 to 30 subunits, such as from 20 to 25 subunits.

In preferred embodiments, each subunit comprises or essentially consists of a nucleotide, or a nucleotide analog. The nucleotide can be a deoxyribonucleic acid comprising a base selected from adenine (A), thymine (T), guanine (G), and cytosine (C), or a ribonucleic acid comprising a base selected from adenine (A), uracil (U), guanine (G), and cytosine (C).

Each of said nucleotides can be linked to a neighbouring nucleotide, or nucleotide analog, by means of a covalent bond, or linked to each neighbouring nucleotide, or nucleotide analog, by means of a covalent bond. The covalent bond is preferably selected from the group consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

It is preferred in one embodiment that at least some of said nucleotides are selected from the group consisting of nucleotide derivatives, including nucleotide derivatives selected from the group consisting of deoxyribonucleic acid derivatives and ribonucleic acid derivatives.

Complementing Element Subunits and Corresponding Coding Element Subunits

When the complementing element subunits are selected from the group consisting of nucleotides, nucleotide derivatives, and nucleotide analogs in which one or more of a base moiety and/or a phosphate moiety and/or a ribose moiety and/or a deoxyribose moiety has been substitutetd by an alternative molecular entity, the coding element subunits capable of interacting with said complementing element subunits preferably comprise or essentially consist of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

It is preferred that each nucleotide derivative is linked to a neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond, or linked to each neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond. The covalent

chemical bond can be selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

Building Blocks, Cleavable Linkers and Selectively Cleavable Linkers

In one aspect there is provided a building block comprising

i) a complementing element capable of specifically recognising a coding element having a recognition group, said complementing element being selected from nucleotides, amino acids, antibodies, antigens, proteins, peptides, and molecules with nucleotide recognizing ability,

ii) at least one functional entity selected from a precursor of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptide, polysulfonamide, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinone, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, and

iii) a linker or selectively cleavable linker separating the functional entity from the complementing element.

The complementing element of the building block is preferably selected from a nucleotide sequence, such as a sequence of from 1 to 8 nucleotides, such as from 1

to 6 nucleotides, for example from 1 to 4 nucleotides, such as from 1 to 3 nucleotides, such as 2 nucleotides or for example 3 nucleotides.

The functional entity can be selected from a precursor of an amino acid selected from alfa amino acids, beta amino acids, gamma amino acids, di-substituted amino acids, poly-substituted amino acids, vinylogous amino acids, N-substituted glycin derivatives and other modified amino acids.

The is also provided a composition of building blocks as defined herein, wherein at least two building blocks of the composition are different.

At least a subset of the plurality of building blocks preferably comprises one complementing element and one functional entity and one linker.

In one embodiment, each building block comprises at least one reactive group type I and/or at least one reactive group type II, including one reactive group type I, two reactive groups type I, one reactive group type II, and two reactive groups type II.

At least one of said reactive groups type II of the functional entity is preferably selected from the group consisting of N-carboxyanhydride (NCA), N-thiocarboxyanhydride (NTA), amine, carboxylic acid, ketone, aldehyde, hydroxyl, thiol, ester, thioester, any conjugated system of double bonds, hydrazine, N-hydroxysuccinimide ester, and epoxide.

In some embodiments, the ractive group type II is an electrophile, a nucleophile, or a radical.

At least a subset of said plurality of building blocks comprises a selectively cleavable linker separating the functional entity from the complementing element, wherein said selectively cleavable linker is not cleaved under conditions resulting in cleavage of cleavable linkers separating the functional entity from the complementing element of building blocks not belonging to the subset of building blocks comprising a selectively cleavable linker. The cleavable linkers of the building blocks are cleaved without cleaving the at least one selectively cleavable linker linking the templated molecule to the complementing template, or to a complementing element, or linking

said templated molecule to a templating element, or to the template that templated the synthesis of the templated molecule.

Linkers and selectively cleavable linkers can be cleaved by e.g. acid, base, a chemical agent, light, electromagnetic radiation, an enzyme, or a catalyst, with the proviso that the cleavage of the cleavable linker does result in the cleavage of the selectively cleavable linker unless this is desirable.

In one embodiment, the length of the linker or selectively cleavable linker is in the range of from about 0.8 Å to about 70 Å, such as in the range of from 0.8 Å to about 60 Å, for example in the range of from 0.8 Å to about 50 Å, such as in the range of from 0.8 Å to about 40 Å, for example in the range of from 0.8 Å to about 30 Å, such as in the range of from 0.8 Å to about 25 Å, for example in the range of from 0.8 Å to about 20 Å, such as in the range of from 0.8 Å to about 18 Å, for example in the range of from 0.8 Å to about 16 Å, such as in the range of from 0.8 Å to about 14 Å, for example in the range of from 0.8 Å to about 12 Å, such as in the range of from 0.8 Å to about 10 Å, for example in the range of from 0.8 Å to about 8 Å, such as in the range of from 0.8 Å to about 7 Å, for example in the range of from 0.8 Å to about 6 Å, such as in the range of from 0.8 Å to about 5 Å, for example in the range of from 0.8 Å to about 4 Å, such as in the range of from 0.8 Å to about 3.5 Å, for example in the range of from 0.8 Å to about 3.0 Å, such as in the range of from 0.8 Å to about 2.5 Å, for example in the range of from 0.8 Å to about 2.0 Å, such as in the range of from 0.8 Å to about 1.5 Å, for example in the range of from 0.8 Å to about 1.0 Å.

In another embodiment, the length of the linker or selectively cleavable linker is in the range of from about 1 Å to about 60 Å, such as in the range of from 1 Å to about 40 Å, for example in the range of from 1 Å to about 30 Å, such as in the range of from 1 Å to about 25 Å, for example in the range of from 1 Å to about 20 Å, such as in the range of from 1 Å to about 18 Å, for example in the range of from 1 Å to about 16 Å, such as in the range of from 1 Å to about 14 Å, for example in the range of from 1 Å to about 12 Å, such as in the range of from 1 Å to about 10 Å, for example in the range of from 1 Å to about 8 Å, such as in the range of from 1 Å to about 7 Å, for example in the range of from 1 Å to about 6 Å, such as in the range of from 1 Å to about 5 Å, for example in the range of from 1 Å to about 4 Å, such as in the range

of from 1.0 Å to about 3.5 Å, for example in the range of from 1.0 Å to about 3.0 Å, such as in the range of from 1.0 Å to about 2.5 Å, for example in the range of from 1.0 Å to about 2.0 Å, such as in the range of from 1.0 Å to about 1.5 Å, for example in the range of from 1.0 Å to about 1.2 Å.

In yet another embodiment, the length of the linker or selectively cleavable linker is in the range of from about 2 Å to about 40 Å, such as in the range of from 2 Å to about 30 Å, such as in the range of from 2 Å to about 25 Å, for example in the range of from 2 Å to about 20 Å, such as in the range of from 2 Å to about 18 Å, for example in the range of from 2 Å to about 16 Å, such as in the range of from 2 Å to about 14 Å, for example in the range of from 2 Å to about 12 Å, such as in the range of from 2 Å to about 10 Å, for example in the range of from 2 Å to about 8 Å, such as in the range of from 2 Å to about 7 Å, for example in the range of from 2 Å to about 6 Å, such as in the range of from 2 Å to about 5 Å, for example in the range of from 2 Å to about 4 Å, such as in the range of from 2.0 Å to about 3.5 Å, for example in the range of from 2.0 Å to about 3.0 Å, such as in the range of from 2.0 Å to about 2.5 Å, for example in the range of from 2.0 Å to about 2.2 Å.

In a further embodiment, the length of the linker or selectively cleavable linker is in the range of from about 4 Å to about 40 Å, such as in the range of from 4 Å to about 30 Å, such as in the range of from 4 Å to about 25 Å, for example in the range of from 4 Å to about 20 Å, such as in the range of from 4 Å to about 18 Å, for example in the range of from 4 Å to about 16 Å, such as in the range of from 4 Å to about 14 Å, for example in the range of from 4 Å to about 12 Å, such as in the range of from 4 Å to about 10 Å, for example in the range of from 4 Å to about 8 Å, such as in the range of from 4 Å to about 7 Å, for example in the range of from 4 Å to about 6 Å, such as in the range of from 4 Å to about 5 Å.

In a still further embodiment, the length of the linker or selectively cleavable linker is in the range of from about 6 Å to about 40 Å, such as in the range of from 6 Å to about 30 Å, such as in the range of from 6 Å to about 25 Å, for example in the range of from 6 Å to about 20 Å, such as in the range of from 6 Å to about 18 Å, for example in the range of from 6 Å to about 16 Å, such as in the range of from 6 Å to about 14 Å, for example in the range of from 6 Å to about 12 Å, such as in the range

of from 6 Å to about 10 Å, for example in the range of from 6 Å to about 8 Å, such as in the range of from 6 Å to about 7 Å.

In yet another embodiment, the length of the linker or selectively cleavable linker is in the range of from about 8 Å to about 40 Å, such as in the range of from 8 Å to about 30 Å, such as in the range of from 8 Å to about 25 Å, for example in the range of from 8 Å to about 20 Å, such as in the range of from 8 Å to about 18 Å, for example in the range of from 8 Å to about 16 Å, such as in the range of from 8 Å to about 14 Å, for example in the range of from 8 Å to about 12 Å, such as in the range of from 8 Å to about 10 Å.

Templated molecules

The templated molecules can be linked - or not linked - to the template having templated the synthesis of the templated molecule.

In one embodiment, the present invention relates to templated molecules comprising or essentially consisting of amino acids selected from the group consisting of α-amino acids, β-amino acids, γ-amino acids, ω-amino acids.

In various preferred embodiments the templated molecule comprises or essentially consists of one or more of natural amino acid residues, of α-amino acids, of monosubstituted α-amino acids, disubstituted α-amino acids, monosubstituted β-amino acids, disubstituted β-amino acids, or trisubstituted β-amino acids, tetrasubstituted β-amino acids, γ-amino acids, ω-amino acids, vinylogous amino acids, and N-substituted glycines.

The above-mentioned templated molecules comprising β-amino acids preferably have a backbone structure comprising or essentially consisting of a cyclohexane-backbone and/or a cyclopentane-backbone.

In other embodiments, the templated molecule comprises or essentially consists of molecules or molecular entities selected from the group of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or

in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptide, polysulfonamide, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinone, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, inlcuding any combination thereof.

Neighbouring residues of the templated molecules according to the invention can be linked by a chemical bond selected from the group of chemical bonds consisting of peptide bonds, sulfonamide bonds, ester bonds, saccharide bonds, carbamate bonds, carbonate bonds, urea bonds, phosphonate bonds,urethane bonds, azatide bonds, peptoid bonds, ether bonds, ethoxy bonds, thioether bonds, single carbon bonds, double carbon bonds, triple carbon bonds, disulfide bonds, sulfide bonds, phosphodiester bonds, oxime bonds, imine bonds, imide bonds, including any combination thereof.

Also, the backbone structure of the templated molecules according to the invention can comprise or essentially consist of a molecular group selected from -NHN(R)CO- ; -NHB(R)CO- ; -NHC(RR')CO- ; -NHC(=CHR)CO- ; -NHC_6H_4CO-; -$NHCH_2CHRCO$-; -$NHCHRCH_2CO$- ; -$COCH_2$- ; -COS- ; -CONR- ; -COO- ; -CSNH- ; -CH_2 NH- ; -CH_2CH_2- ; -CH_2 S- ; -CH_2 SO- ; -CH_2SO_2- ; -$CH(CH_3)$S- ; -CH=CH- ; -NHCO- ; -NHCONH- ; -CONHO- ; -C(=CH_2)CH_2- ; -PO_2^-NH- ; -$PO_2^-CH_2$- ; -$PO_2^-CH_2N^+$- ; -SO_2NH^-- ; and lactams, including any combination thereof.

The precursor is in one embodiment preferably selected from the group of precursors consisting of α-amino acid precursors, β-amino acid precursors, γ-amino acid precursors, and ω-amino acid precursors.

In some embodiment, the templated molecule is an oligomer or a polymer comprising at least one repetitive sequence of functional groups, such as at least

three functional groups repeated at least twice in the templated molecule. The templated molecules also includes molecules wherein any sequence of at least three functional groups occurs only once.

Some preferred templated molecules preferably comprise or essentially consist of at least 2 different functional groups, such as at least 3 different functional groups, for example at least 4 different functional groups, such as at least 5 different functional groups, for example at least 6 different functional groups, such as at least 7 different functional groups, for example at least 8 different functional groups, such as at least 9 different functional groups, for example at least 10 different functional groups, such as more than 10 different functional groups. The functional groups can also be identical.

In one preferred aspect of the invention there is provided a templated molecule comprising a polymer comprising a plurality of covalently linked functional groups each comprising at least one residue, wherein the plurality of residues is preferably from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80,

for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

In another preferred aspect of the invention there is provided a templated molecule comprising a polymer comprising a plurality of covalently linked functional groups each comprising a residue, wherein the covalently linked residues are capable of generating a polymer comprising, exclusively or in combination with additional portions, at least one portion selected from the group of polymer portions consisting of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-

peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopolypeptides, polyamides, vinylogous sulfonamide peptides, polysulfonamides, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinones, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, and wherein the plurality of residues is preferably from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30,

such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

The templated molecule in one embodiment is preferably one, wherein the covalently linked residues are capable of generating a polymer comprising, exclusively or in combination with additional portions selected from the group, at least one portion selected from the group of polymer portions consisting of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, and vinylogous polypeptides.

In one particular embodiment, the templated molecule is one wherein the covalently linked residues are capable of generating a polysaccharaide.

In another aspect there is provided a templated molecule comprising a sequence of functional groups, wherein neighbouring functional groups are linked by a molecular moiety that is not natively associated with said functional groups.

Additional aspect of the present invention relates to i) a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule does not comprise or consist of an α-peptide or a nucleotide, ii) a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule does not comprise or consist of a monosubstituted α-peptide or a nucleotide, and iii) a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule does not comprise or consist of a peptide or a nucleotide.

Compositions of Templated Molecules

The templated molecules according to the invention, including those mentioned herein immediately above, can be present in a composition of templated molecules, wherein said composition comprises a plurality of more than or about 10^3 different templated molecules, such as more than or about 10^4 different templated molecules, for example more than or about 10^5 different templated molecules, such as more than or about 10^6 different templated molecules, for example more than or about 10^7 different templated molecules, such as more than or about 10^8 different templated molecules, for example more than or about 10^9 different templated molecules, such as more than or about 10^{10} different templated molecules, for example more than or about 10^{11} different templated molecules, such as more than or about 10^{12} different templated molecules, for example more than or about 10^{13} different templated molecules, such as more than or about 10^{14} different templated molecules, for example more than or about 10^{15} different templated molecules, such as more than or about 10^{16} different templated molecules, for example more than or about 10^{17} different templated molecules, such as more than or about 10^{18} different templated molecules.

The composition in some embodiments preferably further comprises the template capable of templating each templated molecule, or a subset thereof. Accordingly, in

one preferred aspect of the present invention, there is provided i) a composition comprising a templated molecule and the template capable of templating the templated molecule, or ii) a composition comprising a templated molecule and the template that templated the synthesis of the templated molecule.

Various preferred features of the templated molecules either i) linked to the template capable of templating the synthesis of the templated molecule, or ii) present in a composition further comprising the template capable of templating the synthesis of the templated molecule is listed herein immediately below.

When being present in such compositions, it is preferred that i) the template does not consist exclusively of natural nucleotides, when the templated molecule is a peptide comprising exclusively monosubstituted α-amino acids, ii) the template is not a natural nucleotide, when the templated molecule is a natural α-peptide, iii) the template is not a nucleotide, when the templated molecule is a natural α-peptide, iv) the template is not a nucleotide, when the templated molecule is a monosubstituted α-peptide, v) the template is not a nucleotide, when the templated molecule is an α-peptide, vi) the template is not a natural nucleotide, when the templated molecule is a peptide, and vii) the template is not a nucleotide, when the templated molecule is a peptide.

Templated Molecules Linked to the Template that Templated the Synthesis of the Templated Molecule

In one preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the templated molecule does not comprise or consist of an α-peptide

In another preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or

template that templated the synthesis of the templated molecule, wherein the templated molecule does not comprise a monosubstituted α-peptide.

In yet another preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the templated molecule does not comprise or consist of an α-peptide or a nucleotide.

In a still further aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a natural nucleotide, when the templated molecule is an α-peptide.

In a still further preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template does not consist exclusively of natural nucleotides, when the templated molecule is a peptide comprising exclusively monosubstituted α-amino acids.

In a still further preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a natural nucleotide, when the templated molecule is a natural α-peptide.

In an even further preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule,

wherein the template is not a nucleotide, when the templated molecule is a natural α-peptide.

In a still further preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a nucleotide, when the templated molecule is a monosubstituted α-peptide.

In a still further preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a nucleotide, when the templated molecule is an α-peptide.

In a still further preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a natural nucleotide, when the templated molecule is a peptide.

In a still further preferred aspect of the present invention there is provided a templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a nucleotide, when the templated molecule is a peptide.

The templated molecule can be obtained according to the methods described herein above.

In even further aspects there is provided

i) a templated molecule comprising a sequence of covalently linked building blocks;

ii) a templated molecule comprising a sequence of covalently linked building blocks, wherein the sequence of covalently linked building blocks comprises a sequence of complementing elements forming a complementing template capable of complementing the template that templated the synthesis of the templated molecule, and wherein the templated molecule is linked to the complementing template or template that templated its synthesis; and

iii) a templated molecule according to any of the previous claims, wherein the templated molecule comprises a sequence of functional entities comprising at least one functional group, and optionally at least one reactive group type II, and wherein each functional entity is linked to a complementing element or a template that templated the synthesis of the templated molecule.

Uses of Templated Molecules

The templated molecules according to the present invention can be used for a variety of commercial pruposes.

In one aspect, there is provided a method for screening templated molecules potentially having a predetermined activity, said method comprising the step of providing a target molecule or a target entity, including a surface, and obtaining templated molecules having an affinity for - or an effect on - said target molecule or target entity.

Another aspect relates to a method for assaying an activity potentially associated with a templated molecules, said method comprising the step of providing a target molecule or a target entity, including a surface, and obtaining templated molecules having an affinity for - or an effect on - said target molecule or target entity, and determining the activity of the templated molecule.

Yet another aspect provides a method for selecting complexes or templated molecules having a predetermined activity, said method comprising the step of performing a selection procedure and selecting templated molecules based on predetermined selection criteria.

There is also provided a method for screening a composition of molecules having a predetermined activity comprising:

i) establishing a first composition of templated molecules as described herein, or produced as defined herein by any method for preparing templated molecules,

ii) exposing the first composition to conditions enriching said first composition with templated molecules having the predetermined activity, and

iii) optionally amplifying the templated molecules of the enriched composition obtaining a second composition,

iv) further optionally repeating step ii) to iii), and

v) obtaining a further composition having a higher ratio of templated molecules having the specific predetermined activity.

In one embodiment, the method further comprises a step of mutating the templated molecules, wherein said mutagenesis can take place prior to carrying out step iii), simultaneously with carrying out step iii), or after carrying out step iii). The mutagenesis can be carried out as random or site-directed mutagenesis.

Step iii) of the method preferably comprises a 10^1 to 10^{15}-fold amplification, and steps ii) and iii) can be repeated, such as at least 2 times, 3 times, 5 times, or at least 10 times, such as at least 15 times.

The method can comprise a further step of identifying the templated molecule having the predetermined activity, and said identification can be conducted e.g. by analysing the template and/or complementary template physically or by other means associated with the molecule.

The conditions enriching the first composition can comprise the further providing a binding partner to said templated molecule having the predetermined activity, wherein said binding partner is directly or indirectly immobilised on a support.

The conditions enriching the composition can involve any state of the art method, including any one or more of electrophoretic separation, gelfiltration, immunoprecipitation, isoelectric focusing, centrifugation, and immobilization. The conditions enriching the composition can also comprise the further step of providing cells capable of internalising the templated molecule, or performing any interaction with the templated molecule having the predetermined activity.

The predetermined activity of the templated molecule is preferably an enzymatic activity or a catalytic activity.

In another aspect there is provided a method for amplifying the complementing template or the template that templated the synthesis of the templated molecule having, or potentially having a predetermined activity, said method comprising the step of contacting the template with amplification means, and amplifying the template. The method for amplifying the complementing template or the template that templated the synthesis of the templated molecule having, or potentially having, a predetermined activity, preferably comprises the steps of i) contacting the template with amplification means, and amplifying the template, and ii) obtaining the templated molecule in an at least two-fold increased amount.

In another aspect there is provided a method for altering the sequence of a templated molecule, including generating a templated molecule comprising a novel or altered sequence of functional groups, wherein said method preferably comprises the steps of

i) providing a first complementing template or a first template capable of templating the first templated molecule, or a plurality of such first complementing templates or first templates capable of templating a plurality of first templated molecules,

ii) mutating or modifying the sequence of the first complementing template or the first template, or the plurality of first complementing templates or first templates, and generating a second template or a second complementing template, or a plurality of second templates or second complementing templates,

wherein said second template(s) or complementing template(s) is capable of templating the synthesis of a second templated molecule, or a plurality of second templated molecules,

wherein said second templated molecule(s) comprises a sequence of covalently linked, functional groups that is not identical to the sequence of functional groups of the first templated molecule(s), and optionally

iii) templating by means of said second template(s) or complementing template(s) a second templated molecule, or a plurality of such second templated molecules.

In yet another aspect there is provided a method for altering the sequence of a templated molecule, including generating a templated molecule comprising a novel or altered sequence of functional groups, wherein said method preferably comprises the steps of

i) providing a plurality of first complementing templates or first templates capable of templating a plurality of first templated molecules,

ii) recombining the sequences of the plurality of first complementing templates or first templates, and generating a second template or a second complementing template, or a plurality of second templates or second complementing templates,

wherein said second template(s) or complementing template(s) is capable of templating the synthesis of a second templated molecule, or a plurality of second templated molecules,

wherein said second templated molecule(s) comprises a sequence of covalently linked, functional groups that is not identical to the sequence of functional groups of the first templated molecule(s), and optionally

iii) templating by means of said second template(s) or complementing template(s) a second templated molecule, or a plurality of such second templated molecules.

The methods can preferably comprise the further step of amplifying the complementing template or the template that templated the synthesis of the templated molecule, wherein said amplification step taking place prior to, simultaneously with, or after the step of mutagenesis or recombination.

When mutagenesis is used, it can be used as either site-directed mutagenesis, cassette mutagenesis, chemical mutagenesis, unique site-elimination (USE), error-prone PCR, error-prone DNA shuffling. Mutagenesis preferably involves DNA shuffling and/or any form of recombination including homologous recombination either in vivo or in vitro.

Variants and functional equivalents of templated molecules

The present invention is also directed to any variant and functional equivalent of a templated molecule. The variants and functional equivalents may be obtained by any state-of-the-art-method for modifying templated molecules in the form of polymers, including peptides.

In the context of the templated molecules of the present invention, molecules are said to be homologous if they contain similar backbone structures and/or similar functional groups. Functional groups, or molecular entities of functional groups, are divided into three homology groups: The charged functional groups, the hydrophobic groups, and the hydrophilic groups. When a functional group includes two or three molecular entities belonging to different homology groups, the functional group is said to belong to the two or three different homology groups.

Homology is measured in percent (%). As an example, the sequences AABBCA-CAAA and BBAACACBBB (where A, B and C denotes a functional group belonging to homology group A, B, and C, respectively) are 30 percent homologous.

Claims

1. A method for synthesising a templated molecule comprising a plurality of functional groups, said method comprising the steps of

i) providing at least one template comprising a sequence of n coding elements,

wherein each coding element comprises at least one recognition group capable of recognising a predetermined complementing element, and

wherein n is an integer of more than 1,

ii) providing a plurality of building blocks, wherein each building block comprises

a) at least one complementing element comprising at least one recognition group capable of recognising a predetermined coding element,

b) at least one functional entity comprising at least one functional group and at least one reactive group, and

c) at least one linker separating the at least one functional entity from the at least one complementing element,

iii) obtaining a complementing template by complementing a plurality of predetermined coding elements by contacting each of said coding elements with a complementing element capable of recognising said coding element, and

iv) obtaining a templated molecule comprising a sequence of covalently linked, functional groups by linking, by means of a reaction involving reactive groups, a functional group of at least one functional entity to a functional group of a neighbouring, functional entity,

wherein the templated molecule is capable of being linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, and

wherein the synthesis of the templated molecule does not involve ribosome mediated translation of a nucleic acid.

2. Method of claim 1, wherein the templated molecule is linked by means of a single linker to the complementing template or template that templated the synthesis of the templated molecule.

3. The method of claim 1, wherein the template comprising n coding elements is a linear sequence of coding elements.

4. The method of claim 1, wherein the template comprising n coding elements is branched.

5. The method of claim 1, wherein n preferably has a value of from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6

to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

6. The method of claim 1, wherein the template is attached to a solid or semi-solid support.

7. The method of claim 1, wherein the template comprises or essentially consists of nucleotides selected from the group consisting of deoxyribonucleic acids (DNA), ribonucleic acids (RNA), peptide nucleic acids (PNA), locked nucleic acids (LNA), and morpholinos sequences, including any analog or derivative thereof.

8. The method of claim 1, wherein the template comprises or essentially consists of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof, and wherein the complementing element comprises or essentially consists of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

9. The method of claim 1, wherein the template is amplifyable.

10. The method of claim 1, wherein the template comprises a single strand of coding elements.

11. The method of claim 10, wherein the single strand of coding elements is capable of forming a double helix by hybridization to a complementing template comprising a single strand of complementing elements.

12. The method of any of claims 1 to 11, wherein the template comprises a priming site.

13. The method of claim 1, wherein each coding element is linked to a neighbouring coding element by a covalent chemical bond.

14. The method of claim 1, wherein each coding element is linked to each neighbouring coding element by a covalent chemical bond.

15. The method of any of claims 13 and 14, wherein said covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

16. The method of any of claims 13 and 14, wherein said covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds and phosphorothioate bonds.

17. The method of claim 1, wherein at least one coding element is attached to a solid or semi-solid support.

18. The method of claim 1, wherein the coding elements are selected from the group consisting of nucleotides, including any analog or derivative thereof, amino acids, antibodies, and antigens.

19. The method of claim 18, wherein the coding elements are selected from the group consisting of nucleotides, nucleotide derivatives, and nucleotide analogs, including any combination thereof.

20. The method of claim 19, wherein the coding elements are selected from the group consisting of nucleotides.

21. The method of claim 20, wherein the nucleotides are deoxyribonucleic acids comprising a base selected from adenine (A), thymine (T), guanine (G), and cytosine (C).

22. The method of claim 20, wherein the nucleotides are ribonucleic acids comprising a base selected from adenine (A), uracil (U), guanine (G), and cytosine (C).

23. The method of claim 20, wherein each nucleotide is linked to a neighbouring nucleotide by means of a covalent bond.

24. The method of claim 20, wherein each nucleotide is linked to each neighbouring nucleotide by means of a covalent bond.

25. The method of any of claims 23 and 24, wherein said covalent bond is a phosphodiester bond or a phosphorothioate bond.

26. The method of claim 20, wherein the coding elements are natural and non-natural nucleotides selected from the group consisting of deoxyribonucleic acids.

27. The method of claim 20, wherein the coding elements are natural and non-natural nucleotides selected from the group consisting of ribonucleic acids.

28. The method of claim 18, wherein the coding elements are selected from the group consisting of nucleotides, nucleotide derivatives and nucleotide analogs in which one or more of the base moiety and/or the phosphate moiety and/or the ribose or deoxyribose moiety have been substituted by an alternative molecular entity.

29. The method of claim 28, wherein the complementing elements capable of interacting with said coding elements comprise or essentially consist of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

30. The method of any of claims 26 to 29, wherein each nucleotide is linked to a neighbouring nucleotide by a covalent chemical bond.

31. The method of any of claims 26 to 29, wherein each nucleotide is linked to each neighbouring nucleotide by a covalent chemical bond.

32. The method of any of claims 30 and 31, wherein said covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds and peptide bonds.

33. The method of claim 1, wherein the coding element preferably comprises or essentially consists of from 1 to 100 subunits, such as from 1 to 80 subunits, for example from 1 to 60 subunits, such as from 1 to 40 subunits, for example from 1 to 20 subunits, such as from 1 to 18 subunits, for example from 1 to 16

subunits, such as from 1 to 14 subunits, for example from 1 to 12 subunits, such as from 1 to 10 subunits, for example from 1 to 9 subunits, such as from 1 to 8 subunits, for example from 1 to 7 subunits, such as from 1 to 6 subunits, for example from 1 to 5 subunits, such as from 1 to 4 subunits, for example from 1 to 3 subunits, such as from 1 to 2 subunits, for example 1 subunit, such as from 2 to 100 subunits, such as from 2 to 80 subunits, for example from 2 to 60 subunits, such as from 2 to 40 subunits, for example from 2 to 20 subunits, such as from 2 to 18 subunits, for example from 2 to 16 subunits, such as from 2 to 14 subunits, for example from 2 to 12 subunits, such as from 2 to 10 subunits, for example from 2 to 9 subunits, such as from 2 to 8 subunits, for example from 2 to 7 subunits, such as from 2 to 6 subunits, for example from 2 to 5 subunits, such as from 2 to 4 subunits, for example from 2 to 3 subunits, such as 2 subunits, such as from 3 to 100 subunits, such as from 3 to 80 subunits, for example from 3 to 60 subunits, such as from 3 to 40 subunits, for example from 3 to 20 subunits, such as from 3 to 18 subunits, for example from 3 to 16 subunits, such as from 3 to 14 subunits, for example from 3 to 12 subunits, such as from 3 to 10 subunits, for example from 3 to 9 subunits, such as from 3 to 8 subunits, for example from 3 to 7 subunits, such as from 3 to 6 subunits, for example from 3 to 5 subunits, such as from 3 to 4 subunits, for example 3 subunits, for example from 4 to 100 subunits, such as from 4 to 80 subunits, for example from 4 to 60 subunits, such as from 4 to 40 subunits, for example from 4 to 20 subunits, such as from 4 to 18 subunits, for example from 4 to 16 subunits, such as from 4 to 14 subunits, for example from 4 to 12 subunits, such as from 4 to 10 subunits, for example from 4 to 9 subunits, such as from 4 to 8 subunits, for example from 4 to 7 subunits, such as from 4 to 6 subunits, for example from 4 to 5 subunits, for example 4 subunits, such as from 5 to 100 subunits, such as from 5 to 80 subunits, for example from 5 to 60 subunits, such as from 5 to 40 subunits, for example from 5 to 20 subunits, such as from 5 to 18 subunits, for example from 5 to 16 subunits, such as from 5 to 14 subunits, for example from 5 to 12 subunits, such as from 5 to 10 subunits, for example from 5 to 9 subunits, such as from 5 to 8 subunits, for example from 5 to 7 subunits, such as from 5 to 6 subunits, such as 5 subunits, for example from 6 to 100 subunits, such as from 6 to 80 subunits, for example from 6 to 60 subunits, such as from 6 to 40 subunits, for example from 6 to 20 subunits, such as from 6 to 18 subunits, for example from 6 to 16 subunits, such as from 6 to 14 subunits, for

example from 6 to 12 subunits, such as from 6 to 10 subunits, for example from 6 to 9 subunits, such as from 6 to 8 subunits, for example from 6 to 7 subunits, such as 6 subunits, such as from 7 to 100 subunits, such as from 7 to 80 subunits, for example from 7 to 60 subunits, such as from 7 to 40 subunits, for example from 7 to 20 subunits, such as from 7 to 18 subunits, for example from 7 to 16 subunits, such as from 7 to 14 subunits, for example from 7 to 12 subunits, such as from 7 to 10 subunits, for example from 7 to 9 subunits, such as from 7 to 8 subunits, such as 7 subunits, for example from 8 to 100 subunits, such as from 8 to 80 subunits, for example from 8 to 60 subunits, such as from 8 to 40 subunits, for example from 8 to 20 subunits, such as from 8 to 18 subunits, for example from 8 to 16 subunits, such as from 8 to 14 subunits, for example from 8 to 12 subunits, such as from 8 to 10 subunits, for example from 8 to 9 subunits, for example 8 subunits, such as from 9 to 100 subunits, such as from 9 to 80 subunits, for example from 9 to 60 subunits, such as from 9 to 40 subunits, for example from 9 to 20 subunits, such as from 9 to 18 subunits, for example from 9 to 16 subunits, such as from 9 to 14 subunits, for example from 9 to 12 subunits, such as from 9 to 10 subunits, such as 9 subunits, for example from 10 to 100 subunits, such as from 10 to 80 subunits, for example from 10 to 60 subunits, such as from 10 to 40 subunits, for example from 10 to 20 subunits, such as from 10 to 18 subunits, for example from 10 to 16 subunits, such as from 10 to 14 subunits, for example from 10 to 12 subunits, such as 10 subunits, such as from 11 to 100 subunits, such as from 11 to 80 subunits, for example from 11 to 60 subunits, such as from 11 to 40 subunits, for example from 11 to 20 subunits, such as from 11 to 18 subunits, for example from 11 to 16 subunits, such as from 11 to 14 subunits, for example from 11 to 12 subunits, such as from 12 to 100 subunits, such as from 12 to 80 subunits, for example from 12 to 60 subunits, such as from 12 to 40 subunits, for example from 12 to 20 subunits, such as from 12 to 18 subunits, for example from 12 to 16 subunits, such as from 12 to 14 subunits, for example from 13 to 100 subunits, such as from 13 to 80 subunits, for example from 13 to 60 subunits, such as from 13 to 40 subunits, for example from 13 to 20 subunits, such as from 13 to 18 subunits, for example from 13 to 16 subunits, such as from 13 to 14 subunits, for example from 14 to 100 subunits, such as from 14 to 80 subunits, for example from 14 to 60 subunits, such as from 14 to 40 subunits, for example from 14 to 20 subunits, such as from 14 to 18 subunits, for example from 14 to 16 subunits, such as

from 15 to 100 subunits, such as from 15 to 80 subunits, for example from 15 to 60 subunits, such as from 15 to 40 subunits, for example from 15 to 20 subunits, such as from 15 to 18 subunits, for example from 15 to 16 subunits, such as from 16 to 100 subunits, such as from 16 to 80 subunits, for example from 16 to 60 subunits, such as from 16 to 40 subunits, for example from 16 to 20 subunits, such as from 16 to 18 subunits, for example from 17 to 100 subunits, such as from 17 to 80 subunits, for example from 17 to 60 subunits, such as from 17 to 40 subunits, for example from 17 to 20 subunits, such as from 17 to 18 subunits, for example from 18 to 100 subunits, such as from 18 to 80 subunits, for example from 18 to 60 subunits, such as from 18 to 40 subunits, for example from 18 to 20 subunits, such as from 19 to 100 subunits, such as from 19 to 80 subunits, for example from 19 to 60 subunits, such as from 19 to 40 subunits, for example from 19 to 30 subunits, such as from 19 to 25 subunits, for example from 20 to 100 subunits, such as from 20 to 80 subunits, for example from 20 to 60 subunits, such as from 20 to 40 subunits, for example from 20 to 30 subunits, such as from 20 to 25 subunits.

34. The method of claim 33, wherein each subunit comprises or essentially consists of a nucleotide, or a nucleotide analog.

35. The method of claim 34, wherein each subunit comprises or essentially consists of a nucleotide.

36. The method of claim 35, wherein the nucleotide is a deoxyribonucleic acid comprising a base selected from adenine (A), thymine (T), guanine (G), and cytosine (C).

37. The method of claim 35 , wherein the nucleotide is a ribonucleic acid comprising a base selected from adenine (A), uracil (U), guanine (G), and cytosine (C).

38. The method of claim 35, wherein each nucleotide is linked to a neighbouring nucleotide, or nucleotide analog, by means of a covalent bond.

39. The method of claim 35, wherein each nucleotide is linked to each neighbouring nucleotide, or nucleotide analog, by means of a covalent bond.

40. The method of any of claims 38 and 39, wherein said covalent bond is selected from the group consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

41. The method of claim 34, wherein at least some of said nucleotides are selected from the group consisting of nucleotide derivatives.

42. The method of claim 41, wherein the nucleotide derivatives are selected from the group consisting of deoxyribonucleic acid derivatives and ribonucleic acid derivatives.

43. The method of claim 34, wherein the coding element subunits are selected from the group consisting of nucleotides, nucleotide derivatives and nucleotide analogs in which one or more of a base moiety and/or a phosphate moiety and/or a ribose moiety and/or a deoxyribose moiety have been substituted by an alternative molecular entity.

44. The method of claim 43, wherein the complementing element subunits capable of interacting with said coding element subunits comprise or essentially consist of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

45. The method of claim 41, wherein each nucleotide derivative is linked to a neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond.

46. The method of claim 41, wherein each nucleotide derivative is linked to each neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond.

47. The method of any of claims 45 and 46, wherein said covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

48. The method of claim 1, wherein the complementing template comprising n complementing elements is a linear sequence of coding elements.

49. The method of claim 1, wherein the complementing template comprising n complementing elements is branched.

50. The method of any of claims 48 and 49, wherein n preferably has a value of from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, such as 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, such as 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, such as 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, for example 8, such as 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from

16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

51. The method of claim 1, wherein the complementing template is attached to a solid or semi-solid support.

52. The method of claim 1, wherein the complementing template comprises or essentially consists of nucleotides selected from the group consisting of deoxyribonucleic acids (DNA), ribonucleic acids (RNA), peptide nucleic acids (PNA), locked nucleic acids (LNA), and morpholinos sequences, including any analog or derivative thereof.

53. The method of claim 1, wherein the complementing template comprises or essentially consists of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof, and wherein the corresponding coding element of the template comprises or essentially consists of nucleotides selected from the group

consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

54. The method of claim 1, wherein the complementing template is amplifyable.

55. The method of claim 1, wherein the complementing template comprises a single strand of complementing elements.

56. The method of claim 55, wherein the single strand of complementing elements is capable of forming a double helix by hybridization to a template comprising a single strand of coding elements.

57. The method of any of claims 1 to 56, wherein the complementing template comprises a priming site.

58. The method of claim 1, wherein each complementing element is linked to a neighbouring complementing element by a covalent chemical bond.

59. The method of claim 1, wherein each complementing element is linked to each neighbouring complementing element by a covalent chemical bond.

60. The method of any of claims 58 and 59, wherein said covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

61. The method of any of claims 58 and 59, wherein said covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds and phosphorothioate bonds.

62. The method of claim 1, wherein at least one complementing element is attached to a solid or semi-solid support.

63. The method of claim 1, wherein the complementing elements are selected from the group consisting of nucleotides, including any analog or derivative thereof, amino acids, antibodies, and antigens.

64. The method of claim 63, wherein the complementing elements are selected from the group consisting of nucleotides, nucleotide derivatives, and nucleotide analogs, including any combination thereof.

65. The method of claim 64, wherein the complementing elements are selected from the group consisting of nucleotides.

66. The method of claim 65, wherein the nucleotides are deoxyribonucleic acids comprising a base selected from adenine (A), thymine (T), guanine (G), and cytosine (C).

67. The method of claim 65, wherein the nucleotides are ribonucleic acids comprising a base selected from adenine (A), uracil (U), guanine (G), and cytosine (C).

68. The method of claim 65, wherein each nucleotide is linked to a neighbouring nucleotide, or nucleotide analog, by means of a covalent bond.

69. The method of claim 65, wherein each nucleotide is linked to each neighbouring nucleotide, or nucleotide analog, by means of a covalent bond.

70. The method of any of claims 68 and 69, wherein said covalent bond is a phosphodiester bond or a phosphorothioate bond.

71. The method of claim 63, wherein the complementing elements are natural or non-natural nucleotides selected from the group consisting of deoxyribonucleic acids.

72. The method of claim 63, wherein the complementing elements are natural or non-natural nucleotides selected from the group consisting of ribonucleic acids.

73. The method of claim 63, wherein the complementing elements are selected from the group consisting of nucleotides, nucleotide derivatives and nucleotide analogs in which one or more of a base moiety and/or a phosphate moiety

and/or a ribose and/or a deoxyribose moiety has been substituted by an alternative molecular entity.

74. The method of claim 73, wherein the coding elements capable of interacting with said complementing elements comprise or essentially consist of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

75. The method of any of claims 73 and 74, wherein each nucleotide is linked to a neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond.

76. The method of any of claims 73 and 74, wherein each nucleotide is linked to each neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond.

77. The method of any of claims 75 and 76, wherein said covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

78. The method of claim 1, wherein the complementing element preferably comprises or essentially consists of from 1 to 100 subunits, such as from 1 to 80 subunits, for example from 1 to 60 subunits, such as from 1 to 40 subunits, for example from 1 to 20 subunits, such as from 1 to 18 subunits, for example from 1 to 16 subunits, such as from 1 to 14 subunits, for example from 1 to 12 subunits, such as from 1 to 10 subunits, for example from 1 to 9 subunits, such as from 1 to 8 subunits, for example from 1 to 7 subunits, such as from 1 to 6 subunits, for example from 1 to 5 subunits, such as from 1 to 4 subunits, for example from 1 to 3 subunits, such as from 1 to 2 subunits, for example 1 subunit, such as from 2 to 100 subunits, such as from 2 to 80 subunits, for example from 2 to 60 subunits, such as from 2 to 40 subunits, for example from 2 to 20 subunits, such as from 2 to 18 subunits, for example from 2 to 16 subunits, such as from 2 to 14 subunits, for example from 2 to 12 subunits, such as from 2 to 10 subunits, for example from 2 to 9 subunits, such as from 2 to 8 subunits, for example from 2 to 7 subunits, such as from 2 to 6 subunits, for example from 2 to 5 subunits, such as from 2 to 4 subunits, for example from 2

to 3 subunits, such as 2 subunits, such as from 3 to 100 subunits, such as from 3 to 80 subunits, for example from 3 to 60 subunits, such as from 3 to 40 subunits, for example from 3 to 20 subunits, such as from 3 to 18 subunits, for example from 3 to 16 subunits, such as from 3 to 14 subunits, for example from 3 to 12 subunits, such as from 3 to 10 subunits, for example from 3 to 9 subunits, such as from 3 to 8 subunits, for example from 3 to 7 subunits, such as from 3 to 6 subunits, for example from 3 to 5 subunits, such as from 3 to 4 subunits, for example 3 subunits, for example from 4 to 100 subunits, such as from 4 to 80 subunits, for example from 4 to 60 subunits, such as from 4 to 40 subunits, for example from 4 to 20 subunits, such as from 4 to 18 subunits, for example from 4 to 16 subunits, such as from 4 to 14 subunits, for example from 4 to 12 subunits, such as from 4 to 10 subunits, for example from 4 to 9 subunits, such as from 4 to 8 subunits, for example from 4 to 7 subunits, such as from 4 to 6 subunits, for example from 4 to 5 subunits, for example 4 subunits, such as from 5 to 100 subunits, such as from 5 to 80 subunits, for example from 5 to 60 subunits, such as from 5 to 40 subunits, for example from 5 to 20 subunits, such as from 5 to 18 subunits, for example from 5 to 16 subunits, such as from 5 to 14 subunits, for example from 5 to 12 subunits, such as from 5 to 10 subunits, for example from 5 to 9 subunits, such as from 5 to 8 subunits, for example from 5 to 7 subunits, such as from 5 to 6 subunits, such as 5 subunits, for example from 6 to 100 subunits, such as from 6 to 80 subunits, for example from 6 to 60 subunits, such as from 6 to 40 subunits, for example from 6 to 20 subunits, such as from 6 to 18 subunits, for example from 6 to 16 subunits, such as from 6 to 14 subunits, for example from 6 to 12 subunits, such as from 6 to 10 subunits, for example from 6 to 9 subunits, such as from 6 to 8 subunits, for example from 6 to 7 subunits, such as 6 subunits, such as from 7 to 100 subunits, such as from 7 to 80 subunits, for example from 7 to 60 subunits, such as from 7 to 40 subunits, for example from 7 to 20 subunits, such as from 7 to 18 subunits, for example from 7 to 16 subunits, such as from 7 to 14 subunits, for example from 7 to 12 subunits, such as from 7 to 10 subunits, for example from 7 to 9 subunits, such as from 7 to 8 subunits, such as 7 subunits, for example from 8 to 100 subunits, such as from 8 to 80 subunits, for example from 8 to 60 subunits, such as from 8 to 40 subunits, for example from 8 to 20 subunits, such as from 8 to 18 subunits, for example from 8 to 16 subunits, such as from 8 to 14 subunits, for example from 8 to 12 subunits, such as from 8 to 10 subunits, for

example from 8 to 9 subunits, for example 8 subunits, such as from 9 to 100 subunits, such as from 9 to 80 subunits, for example from 9 to 60 subunits, such as from 9 to 40 subunits, for example from 9 to 20 subunits, such as from 9 to 18 subunits, for example from 9 to 16 subunits, such as from 9 to 14 subunits, for example from 9 to 12 subunits, such as from 9 to 10 subunits, such as 9 subunits, for example from 10 to 100 subunits, such as from 10 to 80 subunits, for example from 10 to 60 subunits, such as from 10 to 40 subunits, for example from 10 to 20 subunits, such as from 10 to 18 subunits, for example from 10 to 16 subunits, such as from 10 to 14 subunits, for example from 10 to 12 subunits, such as 10 subunits, such as from 11 to 100 subunits, such as from 11 to 80 subunits, for example from 11 to 60 subunits, such as from 11 to 40 subunits, for example from 11 to 20 subunits, such as from 11 to 18 subunits, for example from 11 to 16 subunits, such as from 11 to 14 subunits, for example from 11 to 12 subunits, such as from 12 to 100 subunits, such as from 12 to 80 subunits, for example from 12 to 60 subunits, such as from 12 to 40 subunits, for example from 12 to 20 subunits, such as from 12 to 18 subunits, for example from 12 to 16 subunits, such as from 12 to 14 subunits, for example from 13 to 100 subunits, such as from 13 to 80 subunits, for example from 13 to 60 subunits, such as from 13 to 40 subunits, for example from 13 to 20 subunits, such as from 13 to 18 subunits, for example from 13 to 16 subunits, such as from 13 to 14 subunits, for example from 14 to 100 subunits, such as from 14 to 80 subunits, for example from 14 to 60 subunits, such as from 14 to 40 subunits, for example from 14 to 20 subunits, such as from 14 to 18 subunits, for example from 14 to 16 subunits, such as from 15 to 100 subunits, such as from 15 to 80 subunits, for example from 15 to 60 subunits, such as from 15 to 40 subunits, for example from 15 to 20 subunits, such as from 15 to 18 subunits, for example from 15 to 16 subunits, such as from 16 to 100 subunits, such as from 16 to 80 subunits, for example from 16 to 60 subunits, such as from 16 to 40 subunits, for example from 16 to 20 subunits, such as from 16 to 18 subunits, for example from 17 to 100 subunits, such as from 17 to 80 subunits, for example from 17 to 60 subunits, such as from 17 to 40 subunits, for example from 17 to 20 subunits, such as from 17 to 18 subunits, for example from 18 to 100 subunits, such as from 18 to 80 subunits, for example from 18 to 60 subunits, such as from 18 to 40 subunits, for example from 18 to 20 subunits, such as from 19 to 100 subunits, such as from 19 to 80 subunits, for example from 19 to 60 subunits,

such as from 19 to 40 subunits, for example from 19 to 30 subunits, such as from 19 to 25 subunits, for example from 20 to 100 subunits, such as from 20 to 80 subunits, for example from 20 to 60 subunits, such as from 20 to 40 subunits, for example from 20 to 30 subunits, such as from 20 to 25 subunits.

79. The method of claim 78, wherein each subunit comprises or essentially consists of a nucleotide, or a nucleotide analog.

80. The method of claim 79, wherein each subunit comprises or essentially consists of a nucleotide.

81. The method of claim 80, wherein the nucleotide is a deoxyribonucleic acid comprising a base selected from adenine (A), thymine (T), guanine (G), and cytosine (C).

82. The method of claim 80, wherein the nucleotide is a ribonucleic acid comprising a base selected from adenine (A), uracil (U), guanine (G), and cytosine (C).

83. The method of claim 80, wherein each nucleotide is linked to a neighbouring nucleotide, or nucleotide analog, by means of a covalent bond.

84. The method of claim 80, wherein each nucleotide is linked to each neighbouring nucleotide, or nucleotide analog, by means of a covalent bond.

85. The method of claim 84, wherein said covalent bond is selected from the group consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

86. The method of claim 79, wherein at least some of said nucleotides are selected from the group consisting of nucleotide derivatives.

87. The method of claim 86, wherein the nucleotide derivatives are selected from the group consisting of deoxyribonucleic acid derivatives and ribonucleic acid derivatives.

88. The method of claim 79, wherein the complementing element subunits are selected from the group consisting of nucleotides, nucleotide derivatives, and nucleotide analogs in which one or more of a base moiety and/or a phosphate moiety and/or a ribose moiety and/or a deoxyribose moiety has been substitutetd by an alternative molecular entity.

89. The method of claim 88, wherein the coding element subunits capable of interacting with said complementing element subunits comprises or essentially consists of nucleotides selected from the group consisting of DNA, RNA, PNA, LNA and morpholinos sequence, including any analog or derivative thereof.

90. The method of claim 86, wherein each nucleotide derivative is linked to a neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond.

91. The method of claim 86, wherein each nucleotide derivative is linked to each neighbouring nucleotide, or nucleotide analog, by a covalent chemical bond.

92. The method of any of claims 90 and 91, wherein said covalent chemical bond is selected from the group of covalent bonds consisting of phosphodiester bonds, phosphorothioate bonds, and peptide bonds.

93. The method of claim 1, wherein the complementing elements are selected from nucleotides, and the complementing elements are linked enzymatically by using an enzyme selected from the group consisting of template-dependent DNA- and RNA-polymerases, including reverse transcriptases, DNA-ligases and RNA-ligases, ribozymes and deoxyribozymes, including HIV-1 Reverse Transcriptase, AMV Reverse Transcriptase, T7 RNA polymerase, T7 RNA polymerase mutant Y639F, Sequenase, Taq DNA polymerase, Klenow Fragment (Large fragment of DNA polymerase I), DNA-ligase, T7 DNA polymerase, T4 DNA polymerase, T4 DNA Ligase, E. coli RNA polymerase, rTh DNA polymerase, Vent DNA polymerase, Pfu DNA polymerase, Tte DNA polymerase, and ribozymes with ligase or replicase activities.

94. The method of claim 93, wherein the enzyme is selected from the group consisting of HIV-1 Reverse Transcriptase, AMV Reverse Transcriptase, T7

RNA polymerase, T7 RNA polymerase mutant Y639F, Sequenase, Taq DNA polymerase, Klenow Fragment (Large fragment of DNA polymerase I), DNA-ligase, T7 DNA polymerase, T4 DNA polymerase, and T4 DNA Ligase.

95. The method of any of claims 93 and 94, wherein the nucleotides form a template or complementing template upon incorporation.

96. The method of claim 1, wherein the complementing elements are selected from nucleotides, and the complementing elements are linked by using a chemical agent, pH change, light, a catalyst, radiation, such as electromagnetic radiation, or by spontaneous coupling when being brought into reactive contact with each other.

97. Method of claim 1, wherein at least a subset of said plurality of building blocks preferably comprises one complementing element and/or one functional entity and/or one linker.

98. The method of claim 1, wherein a subset of said plurality of building blocks comprises a selectively cleavable linker separating the functional entity from the complementing element, wherein said selectively cleavable linker is not cleaved under conditions resulting in cleavage of cleavable linkers separating the functional entity from the complementing element of building blocks not belonging to the subset of building blocks comprising a selectively cleavable linker.

99. The method of claim 98, wherein the cleavable linkers are cleaved, and wherein the at least one selectively cleavable linker is not cleaved, and wherein the templated molecule is linked to the template and/or to the complementing element by means of said at least one selectively cleavable linker.

100. The method of claim 99, wherein the linkers are cleaved by acid, base, a chemical agent, light, electromagnetic radiation, an enzyme, or a catalyst.

101. The method of claim 1, wherein the length of the linker or selectively cleavable linker is in the range of from about 0.8 Å to about 70 Å, such as in the

range of from 0.8 Å to about 60 Å, for example in the range of from 0.8 Å to about 50 Å, such as in the range of from 0.8 Å to about 40 Å, for example in the range of from 0.8 Å to about 30 Å, such as in the range of from 0.8 Å to about 25 Å, for example in the range of from 0.8 Å to about 20 Å, such as in the range of from 0.8 Å to about 18 Å, for example in the range of from 0.8 Å to about 16 Å, such as in the range of from 0.8 Å to about 14 Å, for example in the range of from 0.8 Å to about 12 Å, such as in the range of from 0.8 Å to about 10 Å, for example in the range of from 0.8 Å to about 8 Å, such as in the range of from 0.8 Å to about 7 Å, for example in the range of from 0.8 Å to about 6 Å, such as in the range of from 0.8 Å to about 5 Å, for example in the range of from 0.8 Å to about 4 Å, such as in the range of from 0.8 Å to about 3.5 Å, for example in the range of from 0.8 Å to about 3.0 Å, such as in the range of from 0.8 Å to about 2.5 Å, for example in the range of from 0.8 Å to about 2.0 Å, such as in the range of from 0.8 Å to about 1.5 Å, for example in the range of from 0.8 Å to about 1.0 Å.

102. The method of claim 1, wherein the length of the linker or selectively cleavable linker is in the range of from about 1 Å to about 60 Å, such as in the range of from 1 Å to about 40 Å, for example in the range of from 1 Å to about 30 Å, such as in the range of from 1 Å to about 25 Å, for example in the range of from 1 Å to about 20 Å, such as in the range of from 1 Å to about 18 Å, for example in the range of from 1 Å to about 16 Å, such as in the range of from 1 Å to about 14 Å, for example in the range of from 1 Å to about 12 Å, such as in the range of from 1 Å to about 10 Å, for example in the range of from 1 Å to about 8 Å, such as in the range of from 1 Å to about 7 Å, for example in the range of from 1 Å to about 6 Å, such as in the range of from 1 Å to about 5 Å, for example in the range of from 1 Å to about 4 Å, such as in the range of from 1.0 Å to about 3.5 Å, for example in the range of from 1.0 Å to about 3.0 Å, such as in the range of from 1.0 Å to about 2.5 Å, for example in the range of from 1.0 Å to about 2.0 Å, such as in the range of from 1.0 Å to about 1.5 Å, for example in the range of from 1.0 Å to about 1.2 Å.

103. The method of claim 1, wherein the length of the linker or selectively cleavable linker is in the range of from about 2 Å to about 40 Å, such as in the range of from 2 Å to about 30 Å, such as in the range of from 2 Å to about 25 Å,

for example in the range of from 2 Å to about 20 Å, such as in the range of from 2 Å to about 18 Å, for example in the range of from 2 Å to about 16 Å, such as in the range of from 2 Å to about 14 Å, for example in the range of from 2 Å to about 12 Å, such as in the range of from 2 Å to about 10 Å, for example in the range of from 2 Å to about 8 Å, such as in the range of from 2 Å to about 7 Å, for example in the range of from 2 Å to about 6 Å, such as in the range of from 2 Å to about 5 Å, for example in the range of from 2 Å to about 4 Å, such as in the range of from 2.0 Å to about 3.5 Å, for example in the range of from 2.0 Å to about 3.0 Å, such as in the range of from 2.0 Å to about 2.5 Å, for example in the range of from 2.0 Å to about 2.2 Å.

104. The method of claim 1, wherein the length of the linker or selectively cleavable linker is in the range of from about 4 Å to about 40 Å, such as in the range of from 4 Å to about 30 Å, such as in the range of from 4 Å to about 25 Å, for example in the range of from 4 Å to about 20 Å, such as in the range of from 4 Å to about 18 Å, for example in the range of from 4 Å to about 16 Å, such as in the range of from 4 Å to about 14 Å, for example in the range of from 4 Å to about 12 Å, such as in the range of from 4 Å to about 10 Å, for example in the range of from 4 Å to about 8 Å, such as in the range of from 4 Å to about 7 Å, for example in the range of from 4 Å to about 6 Å, such as in the range of from 4 Å to about 5 Å.

105. The method of claim 1, wherein the length of the linker or selectively cleavable linker is in the range of from about 6 Å to about 40 Å, such as in the range of from 6 Å to about 30 Å, such as in the range of from 6 Å to about 25 Å, for example in the range of from 6 Å to about 20 Å, such as in the range of from 6 Å to about 18 Å, for example in the range of from 6 Å to about 16 Å, such as in the range of from 6 Å to about 14 Å, for example in the range of from 6 Å to about 12 Å, such as in the range of from 6 Å to about 10 Å, for example in the range of from 6 Å to about 8 Å, such as in the range of from 6 Å to about 7 Å.

106. The method of claim 1, wherein the length of the linker or selectively cleavable linker is in the range of from about 8 Å to about 40 Å, such as in the range of from 8 Å to about 30 Å, such as in the range of from 8 Å to about 25 Å, for example in the range of from 8 Å to about 20 Å, such as in the range of from

8 Å to about 18 Å, for example in the range of from 8 Å to about 16 Å, such as in the range of from 8 Å to about 14 Å, for example in the range of from 8 Å to about 12 Å, such as in the range of from 8 Å to about 10 Å.

107. The method of claim 1, wherein the templated molecule is an oligomer or a polymer comprising at least one repetitive sequence of functional groups.

108. Method of claim 107, wherein the sequence of at least three functional groups is repeated at least twice in the templated molecule.

109. Method of claim 107, wherein any sequence of at least three functional groups in the templated molecule occurs only once.

110. The method of claim 1, wherein the templated molecule comprises or essentially consists of amino acids selected from the group consisting of α-amino acids, β-amino acids, γ-amino acids, ω-amino acids.

111. The method of claim 1, wherein the templated molecule comprises or essentially consists of natural amino acid residues.

112. The method of claim 1, wherein the templated molecule comprises or essentially consists of α-amino acids.

113. The method of claim 1, wherein the templated molecule comprises or essentially consists of monosubstituted α-amino acids.

114. The method of claim 1, wherein the templated molecule comprises or essentially consists of disubstituted α-amino acids.

115. The method of claim 1, wherein the templated molecule comprises or essentially consists of monosubstituted β-amino acids.

116. The method of claim 1, wherein the templated molecule comprises or essentially consists of disubstituted β-amino acids.

117. The method of claim 1, wherein the templated molecule comprises or essentially consists of trisubstituted β-amino acids.

118. The method of claim 1, wherein the templated molecule comprises or essentially consists of tetrasubstituted β-amino acids.

119. The method of any of claims 115 to 118, wherein the backbone structure of said β-amino acids comprises or essentially consists of a cyclohexane-backbone and/or a cyclopentane-backbone.

120. The method of claim 1, wherein the templated molecule comprises or essentially consists of γ-amino acids.

121. The method of claim 1, wherein the templated molecule comprises or essentially consists of ω-amino acids.

122. The method of claim 1, wherein the templated molecule comprises or essentially consists of vinylogous amino acids.

123. The method of claim 1, wherein the templated molecule comprises or essentially consists of N-substituted glycines.

124. The method of claim 1, wherein the templated molecule comprises or essentially consists of molecules or molecular entities selected from the group of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopolypeptides, polyamides, vinylogous sulfonamide peptide, polysulfonamide, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinone, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes,

polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, inlcuding any combination thereof.

125. The method of claim 1, wherein neighbouring residues of the templated molecule is linked by a chemical bond selected from the group of chemical bonds consisting of peptide bonds, sulfonamide bonds, ester bonds, saccharide bonds, carbamate bonds, carbonate bonds, urea bonds, phosphonate bonds,urethane bonds, azatide bonds, peptoid bonds, ether bonds, ethoxy bonds, thioether bonds, single carbon bonds, double carbon bonds, triple carbon bonds, disulfide bonds, sulfide bonds, phosphodiester bonds, oxime bonds, imine bonds, imide bonds, including any combination thereof.

126. The method of claim 1, wherein the backbone structure of said templated molecule comprises or essentially consists of a molecular group selected from -NHN(R)CO- ; -NHB(R)CO- ; -NHC(RR')CO- ; -NHC(=CHR)CO- ; $-NHC_6H_4CO-$; $-NHCH_2CHRCO-$; $-NHCHRCH_2CO-$; $-COCH_2-$; -COS- ; -CONR- ; -COO- ; -CSNH- ; $-CH_2NH-$; $-CH_2CH_2-$; $-CH_2S-$; $-CH_2SO-$; $-CH_2SO_2-$; $-CH(CH_3)S-$; -CH=CH- ; -NHCO- ; -NHCONH- ; -CONHO- ; $-C(=CH_2)CH_2-$; $-PO_2^-NH-$; $-PO_2^-CH_2-$; $-PO_2^-CH_2N^+-$; $-SO_2NH^-$- ; and lactams.

127. The method of claim 1, wherein the precursor is selected from the group of precursors selected from α-amino acid precursors, β-amino acid precursors, γ-amino acid precursors, and ω-amino acid precursors.

128. The method of claim 1, wherein the templated molecule comprises or essentially consists of at least 2 different functional groups, such as at least 3 different functional groups, for example at least 4 different functional groups, such as at least 5 different functional groups, for example at least 6 different functional groups, such as at least 7 different functional groups, for example at least 8 different functional groups, such as at least 9 different functional groups, for example at least 10 different functional groups, such as more than 10 different functional groups.

129. The method of claim 128, wherein the functional groups are identical.

130. The method of claim 1, wherein each building block comprises at least one reactive group type I and at least one reactive group type II.

131. The method of claim 130, wherein each building block comprises one reactive group type I.

132. The method of claim 130, wherein each building block comprises two reactive groups type I.

133. The method of claim 130, wherein each building block comprises one reactive group type II.

134. The method of claim 130, wherein each building block comprises two reactive groups type II.

135. The method of any of claims 130 to 134, wherein the at least one reactive group type II of the functional entity is selected from the group consisting of N-carboxyanhydride (NCA), N-thiocarboxyanhydride (NTA), amine, carboxylic acid, ketone, aldehyde, hydroxyl, thiol, ester, thioester, any conjugated system of double bonds, hydrazine, N-hydroxysuccinimide ester, and epoxide.

136. The method of claim 130, wherein the reactive group type II is an electrophile.

137. The method of claim 130, wherein the reactive group type II is a nucleophile.

138. The method of claim 130, wherein the reactive group type II is a radical.

139. The method of any of the preceding claims comprising the further step of releasing the template or complementing template from the templated

molecule, and obtaining a templated molecule that is not linked to the complementing template or template that templated the synthesis of the templated molecule.

140. A templated molecule comprising a polymer comprising a plurality of covalently linked functional groups each comprising at least one residue, wherein the plurality of residues is preferably from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example

from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

141. A templated molecule comprising a polymer comprising a plurality of covalently linked functional groups each comprising a residue, wherein the covalently linked residues are capable of generating a polymer comprising, exclusively or in combination with additional portions, at least one portion selected from the group of polymer portions consisting of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptides, polysulfonamides, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo

pyrrolinones, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, and wherein the plurality of residues is preferably from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80, for example from 10 to 60, such as from 10 to 40, for example from 10 to 30, such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as from 16 to 80, for example from 16 to 60, such as from 16 to 40,

for example from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30, such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to 60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40 to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60, for example from 45 to 50, such as from 50 to 100, for example from 50 to 80, such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example from 80 to 90, such as from 90 to 100.

142. The templated molecule according to claim 141, wherein the covalently linked residues are capable of generating a polymer comprising, exclusively or in combination with additional portions selected from the group, at least one portion selected from the group of polymer portions consisting of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, and vinylogous polypeptides.

143. The templated molecule according to claim 141, wherein the covalently linked residues are capable of generating a polysaccharaide.

144. A templated molecule comprising a sequence of functional groups, wherein neighbouring functional groups are linked by a molecular moiety that is not natively associated with said functional groups.

145. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule does not comprise or consist of an α-peptide or a nucleotide.

146. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule does not comprise or consist of a monosubstituted α-peptide or a nucleotide.

147. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule does not comprise or consist of a peptide or a nucleotide.

148. A composition of templated molecules, wherein said composition comprises a plurality of more than or about 10^3 different templated molecules, such as more than or about 10^4 different templated molecules, for example more than or about 10^5 different templated molecules, such as more than or about 10^6 different templated molecules, for example more than or about 10^7 different templated molecules, such as more than or about 10^8 different templated molecules, for example more than or about 10^9 different templated molecules, such as more than or about 10^{10} different templated molecules, for example more than or about 10^{11} different templated molecules, such as more than or about 10^{12} different templated molecules, for example more than or about 10^{13} different templated molecules, such as more than or about 10^{14} different templated molecules, for example more than or about 10^{15} different templated molecules, such as more than or about 10^{16} different templated molecules, for example more than or about 10^{17} different templated molecules, such as more than or about 10^{18} different templated molecules.

149. The composition according to claim 148, wherein the plurality of templated molecules is selected from the group of templated molecules defined in any of claims 140 to 147.

150. The composition according to any of claims 148 and 149, wherein said composition further comprises the template capable of templating each templated molecule, or a subset thereof.

151. A composition comprising a templated molecule and the template capable of templating the templated molecule.

152. A composition comprising a templated molecule and the template that templated the synthesis of the templated molecule.

153. The composition according to any of claims 148 to 152, wherein the template is not a natural nucleotide, when the templated molecule is an α-peptide.

154. The composition according to claim 148 to 152, wherein the template does not consist exclusively of natural nucleotides, when the templated molecule is a peptide comprising exclusively monosubstituted α-amino acids.

155. The composition according to claim 148 to 152, wherein the template is not a natural nucleotide, when the templated molecule is a natural α-peptide.

156. The composition according to claim 148 to 152, wherein the template is not a nucleotide, when the templated molecule is a natural α-peptide.

157. The composition according to claim 148 to 152, wherein the template is not a nucleotide, when the templated molecule is a monosubstituted α-peptide.

158. The composition according to claim 148 to 152, wherein the template is not a nucleotide, when the templated molecule is an α-peptide.

159. The composition according to claim 148 to 152, wherein the template is not a natural nucleotide, when the templated molecule is a peptide.

160. The composition according to claim 148, to 152, wherein the template is not a nucleotide, when the templated molecule is a peptide.

161. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the templated molecule does not comprise or consist of an α-peptide

162. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the templated molecule does not comprise a monosubstituted α-peptide.

163. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the templated molecule does not comprise or consist of an α-peptide or a nucleotide.

164. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a natural nucleotide, when the templated molecule is an α-peptide.

165. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template does not consist exclusively of natural nucleotides, when the templated molecule is a peptide comprising exclusively monosubstituted α-amino acids.

166. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the

templated molecule, wherein the template is not a natural nucleotide, when the templated molecule is a natural α-peptide.

167. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a nucleotide, when the templated molecule is a natural α-peptide.

168. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a nucleotide, when the templated molecule is a monosubstituted α-peptide.

169. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a nucleotide, when the templated molecule is an α-peptide.

170. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a natural nucleotide, when the templated molecule is a peptide.

171. A templated molecule comprising a sequence of covalently linked, functional groups, wherein the templated molecule is linked by means of a linker to the complementing template or template that templated the synthesis of the templated molecule, wherein the template is not a nucleotide, when the templated molecule is a peptide.

172. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule is an oligomer or a polymer comprising at least one repetitive sequence of functional groups.

173. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the sequence of at least three functional groups is repeated at least twice in the templated molecule.

174. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein any sequence of at least three functional groups in the templated molecule occurs only once.

175. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of amino acids selected from the group consisting of α-amino acids, β-amino acids, γ-amino acids, ω-amino acids.

176. The templated molecule according to any of claims 140 to 164, wherein the templated molecule comprises or essentially consists of natural amino acid residues.

177. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of α-amino acids.

178. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of monosubstituted α-amino acids.

179. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of disubstituted α-amino acids.

180. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of monosubstituted β-amino acids.

181. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of disubstituted β-amino acids.

182. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of trisubstituted β-amino acids.

183. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of tetrasubstituted β-amino acids.

184. The method of any of claims 176 to 179, wherein the backbone structure of said β-amino acids comprises or essentially consists of a cyclohexane-backbone and/or a cyclopentane-backbone.

185. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of γ-amino acids.

186. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of ω-amino acids.

187. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of vinylogous amino acids.

188. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of N-substituted glycines.

189. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of molecules or molecular entities selected from the group of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptide, polysulfonamide, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinone, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, inlcuding any combination thereof.

190. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein neighbouring residues of the templated molecule is linked by a chemical bond selected from the group of chemical bonds consisting of peptide bonds, sulfonamide bonds, ester bonds, saccharide bonds, carbamate bonds, carbonate bonds, urea bonds, phosphonate bonds,urethane bonds, azatide bonds, peptoid bonds, ether bonds, ethoxy bonds, thioether bonds, single carbon bonds, double carbon bonds, triple carbon bonds, disulfide bonds, sulfide bonds, phosphodiester bonds, oxime bonds, imine bonds, imide bonds, including any combination thereof.

191. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the backbone structure of said templated molecule comprises or essentially consists of a molecular group selected from -NHN(R)CO- ; -NHB(R)CO- ; -NHC(RR')CO- ; -NHC(=CHR)CO- ; -NHC$_6$ H$_4$ CO-; -NHCH$_2$ CHRCO-; -NHCHRCH$_2$ CO- ; -COCH$_2$- ; -COS- ; -CONR- ; -COO- ; -CSNH- ; -CH$_2$ NH- ; -CH$_2$CH$_2$- ; -CH$_2$ S- ; -CH$_2$ SO- ;

$-CH_2SO_2-$; $-CH(CH_3)S-$; -CH=CH- ; -NHCO- ; -NHCONH- ; -CONHO- ; $-C(=CH_2)CH_2-$; $-PO_2^-NH-$; $-PO_2^-CH_2-$; $-PO_2^-CH_2N^+-$; $-SO_2NH^-$; and lactams, including any combination thereof.

192. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the precursor is selected from the group of precursors selected from α-amino acid precursors, β-amino acid precursors, γ-amino acid precursors, and ω-amino acid precursors.

193. The templated molecule according to any of claims 140 to 147, and 161 to 171, wherein the templated molecule comprises or essentially consists of at least 2 different functional groups, such as at least 3 different functional groups, for example at least 4 different functional groups, such as at least 5 different functional groups, for example at least 6 different functional groups, such as at least 7 different functional groups, for example at least 8 different functional groups, such as at least 9 different functional groups, for example at least 10 different functional groups, such as more than 10 different functional groups.

194. The templated molecule according to claim 193, wherein the functional groups are identical.

195. The templated molecule according to any of claims 140 to 147, and 161 to 194, wherein the templated molecule is obtained by a method according to any of claims 1 to 139.

196. A templated molecule comprising a sequence of covalently linked building blocks.

197. A templated molecule comprising a sequence of covalently linked building blocks, wherein the sequence of covalently linked building blocks comprises a sequence of complementing elements forming a complementing template capable of complementing the template that templated the synthesis of the templated molecule, and wherein the templated molecule is linked to the complementing template or template that templated its synthesis.

198. A templated molecule according to any of the previous claims, wherein the templated molecule comprises a sequence of functional entities comprising at least one functional group, and optionally at least one reactive group type II, and wherein each functional entity is linked to a complementing element or a template that templated the synthesis of the templated molecule.

199. A method for screening templated molecules potentially having a predetermined activity, said method comprising the step of providing a target molecule or a target entity, including a surface, and obtaining templated molecules having an affinity for - or an effect on - said target molecule or target entity.

200. A method for assaying an activity potentially associated with a templated molecules, said method comprising the step of providing a target molecule or a target entity, including a surface, and obtaining templated molecules having an affinity for - or an effect on - said target molecule or target entity, and determining the activity of the templated molecule.

201. A method for selecting complexes or templated molecules having a predetermined activity, said method comprising the step of performing a selection procedure and selecting templated molecules based on predetermined selection criteria.

202. A method for screening a composition of molecules having a predetermined activity comprising:

i) establishing a first composition of templated molecules, said molecules being defined as in any of the claims 140 to 147, and 161 to 198, or produced as defined in any of the claims 1 to 139,

ii) exposing the first composition to conditions enriching said first composition with templated molecules having the predetermined activity, and

iii) optionally amplifying the templated molecules of the enriched composition obtaining a second composition,

iv) further optionally repeating step ii) to iii), and

v) obtaining a further composition having a higher ratio of templated molecules having the specific predetermined activity.

203. The method of claim 202, further comprising a step of mutating the templated molecules, wherein said mutagenesis can take place prior to carrying out step iii). simultaneously with carrying out step iii), or after carrying out step iii).

204. The method of claim 203, wherein the mutagenesis is carried out as random or site-directed mutagenesis.

205. The method of claim 202, wherein step iii) comprises a 10^1 to 10^{15}-fold amplification.

206. The method of claim 202, wherein the steps ii) and iii) are repeated at least 2, 3, 5 times, such as at least 10 times, such as at least 15 times.

207. The method of claim 202, further comprising a step of identification of the templated molecule having the predetermined activity.

208. The method of claim 207, wherein the identification is conducted by analysing the template and/or complementary template physically or by other means associated with the molecule.

209. The method of claim 202, wherein the conditions enriching the composition comprises providing a binding partner to said templated molecule having the predetermined activity, said binding partner being directly or indirectly immobilised on a support.

210. The method of claim 202, wherein the conditions enriching the composition involves any one or more of electrophoretic separation, gelfiltration, immunoprecipitation, isoelectric focusing, centrifugation, and immobilization.

211. The method of claim 202, wherein the predetermined activity is an enzymatic activity or a catalytic activity.

212. The method of claim 202, wherein the conditions enriching the composition comprises providing cells capable of internalising the templated molecule, or performing an interaction with the templated molecule having the predetermined activity.

213. A method for amplifying the complementing template or the template that templated the synthesis of the templated molecule having, or potentially having a predetermined activity, said method comprising the step of contacting the template with amplification means, and amplifying the template.

214. A method for amplifying the complementing template or the template that templated the synthesis of the templated molecule having, or potentially having, a predetermined activity, said method comprising the steps of i) contacting the template with amplification means, and amplifying the template, and ii) obtaining the templated molecule in an at least two-fold increased amount.

215. A method for altering the sequence of a templated molecule, including generating a templated molecule comprising a novel or altered sequence of functional groups, wherein said method preferably comprises the steps of

i) providing a first complementing template or a first template capable of templating the first templated molecule, or a plurality of such first complementing templates or first templates capable of templating a plurality of first templated molecules,

ii) mutating or modifying the sequence of the first complementing template or the first template, or the plurality of first complementing templates or

first templates, and generating a second template or a second complementing template, or a plurality of second templates or second complementing templates,

wherein said second template(s) or complementing template(s) is capable of templating the synthesis of a second templated molecule, or a plurality of second templated molecules,

wherein said second templated molecule(s) comprises a sequence of covalently linked, functional groups that is not identical to the sequence of functional groups of the first templated molecule(s), and optionally

iii) templating by means of said second template(s) or complementing template(s) a second templated molecule, or a plurality of such second templated molecules.

216. A method for altering the sequence of a templated molecule, including generating a templated molecule comprising a novel or altered sequence of functional groups, wherein said method preferably comprises the steps of

i) providing a plurality of first complementing templates or first templates capable of templating a plurality of first templated molecules,

ii) recombining the sequences of the plurality of first complementing templates or first templates, and generating a second template or a second complementing template, or a plurality of second templates or second complementing templates,

wherein said second template(s) or complementing template(s) is capable of templating the synthesis of a second templated molecule, or a plurality of second templated molecules,

wherein said second templated molecule(s) comprises a sequence of covalently linked, functional groups that is not identical to the sequence of functional groups of the first templated molecule(s), and optionally

iii) templating by means of said second template(s) or complementing template(s) a second templated molecule, or a plurality of such second templated molecules.

217. The method of any of claims 215 and 216, comprising the further step of amplifying the complementing template or the template that templated the synthesis of the templated molecule, said amplification step taking place prior to, simultaneously with, or after the step of mutagenesis or recombination.

218. The method of claim 215, wherein the mutagenesis is conducted as site-directed mutagenesis, cassette mutagenesis, chemical mutagenesis, unique site-elimination (USE), error-prone PCR, error-prone DNA shuffling.

219. The method of claim 215, wherein the mutagenesis is conducted as DNA shuffling or any form of recombination including homologous recombination either in vivo or in vitro.

220. A building block comprising

i) a complementing element capable of specifically recognising a coding element having a recognition group, said complementing element being selected from nucleotides, amino acids, antibodies, antigens, proteins, peptides, and molecules with nucleotide recognizing ability,

ii) at least one functional entity selected from a precursor of α-peptides, β-peptides, γ-peptides, ω-peptides, mono-, di- and tri-substituted α-peptides, β-peptides, γ-peptides, ω-peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptide, polysulfonamide, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinone,

polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, and

iii) a linker separating the functional entity from the complementing element.

221. The building block according to claim 220, wherein the complementing element is selected from a nucleotide sequence, such as a sequence of from 1-4 nucleotides, such as from 1-3 nucleotides, such as 2 nucleotides or 3 nucleotides.

222. The building block according to claim 220, wherein the functional entity is selected from a precursor of an amino acid selected from alfa amino acids, beta amino acids, gamma amino acids, di-substituted amino acids, poly-substituted amino acids, vinylogous amino acids, N-substituted glycin derivatives and other modified amino acids.

223. A composition of building blocks as defined in any of claims 1 to 139, and claims 220 to 222, wherein at least two building blocks are different.

Figure 1. Chemical Display – Principle.

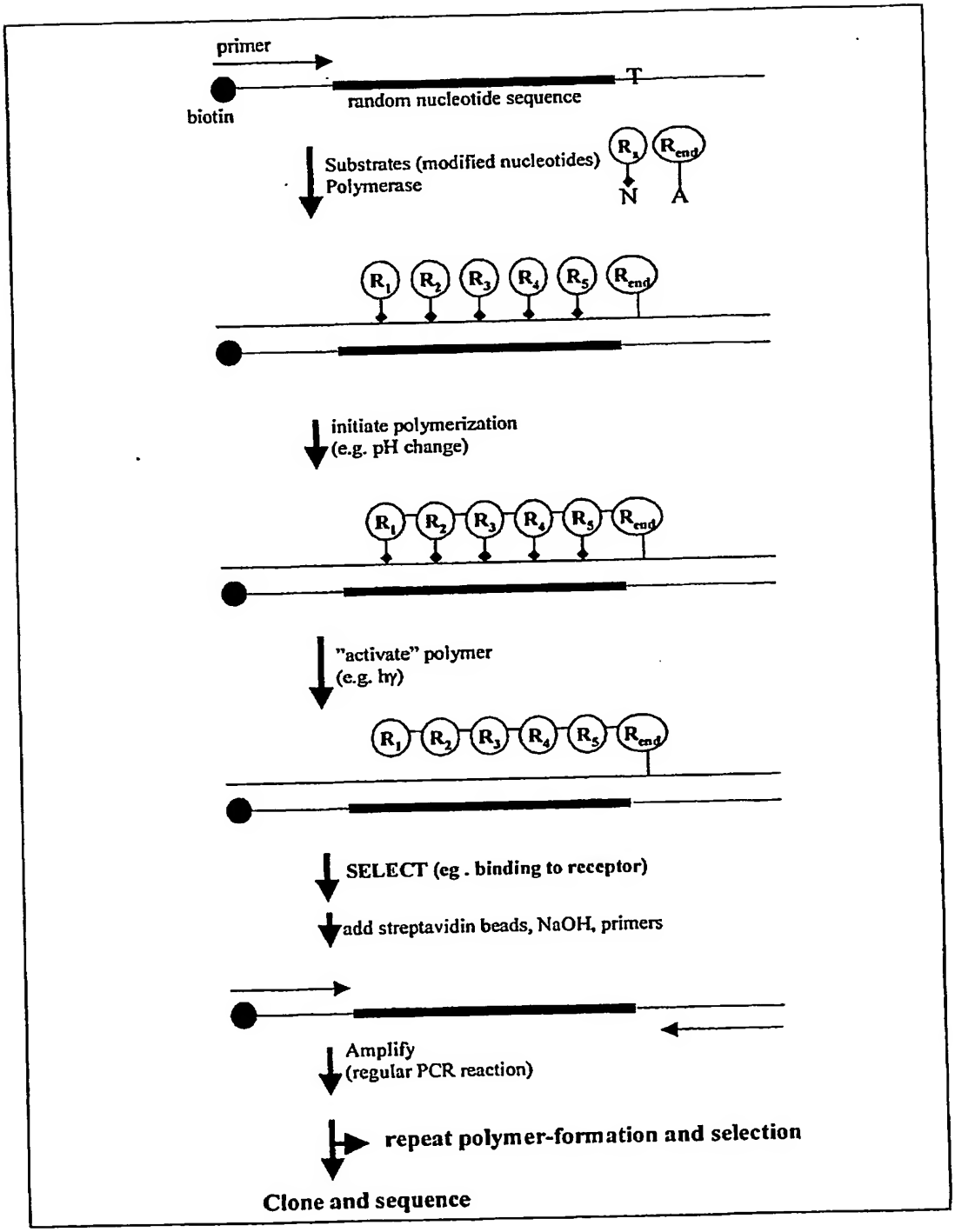


Figure 2a. An expanded set of base pairs.

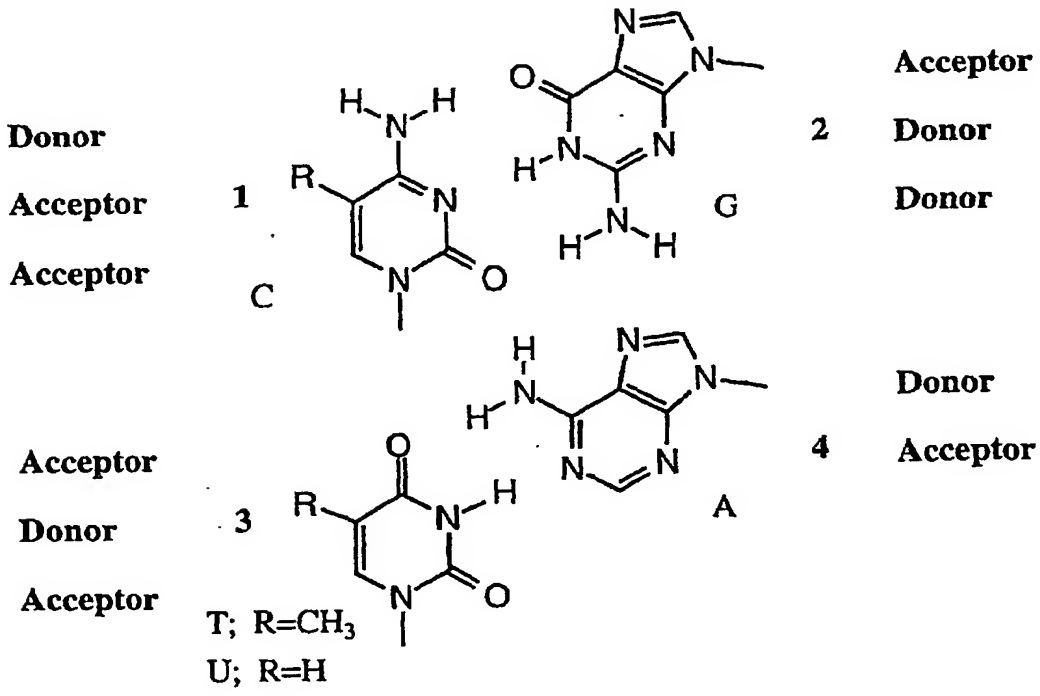


Figure 2b. An expanded set of base pairs.

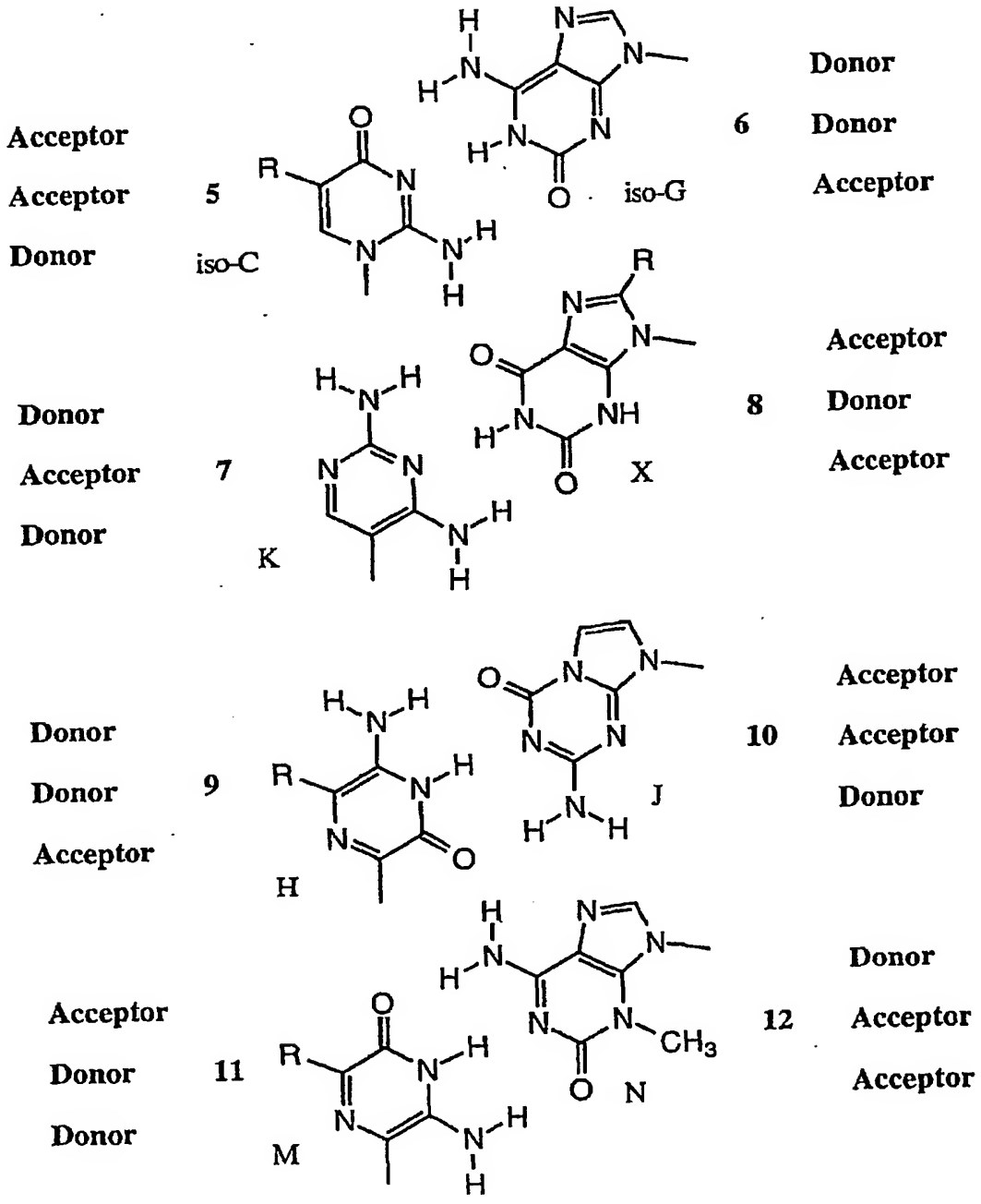


Figure 3. A monomer building block.

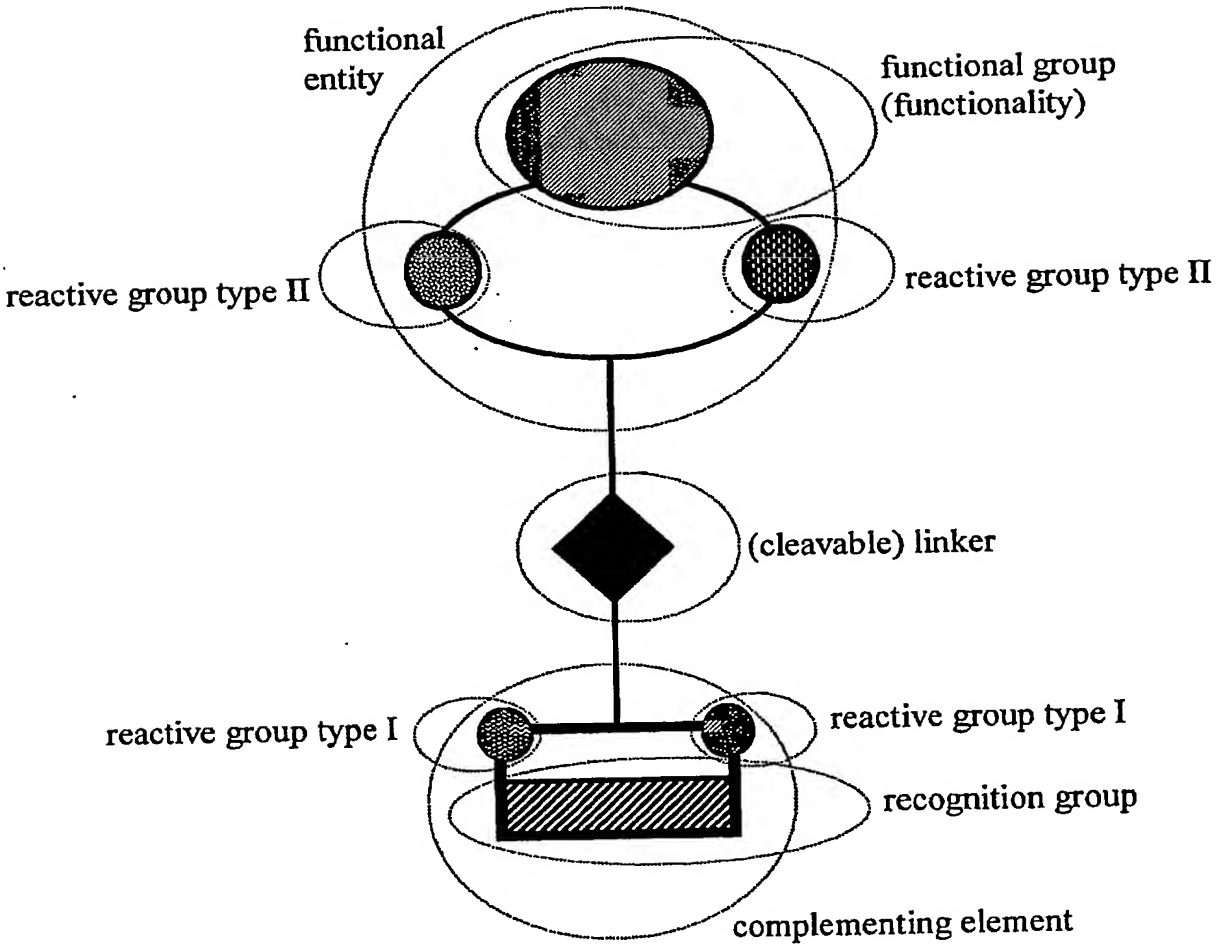


Figure 4. A monomer building block with only one reactive group type II.

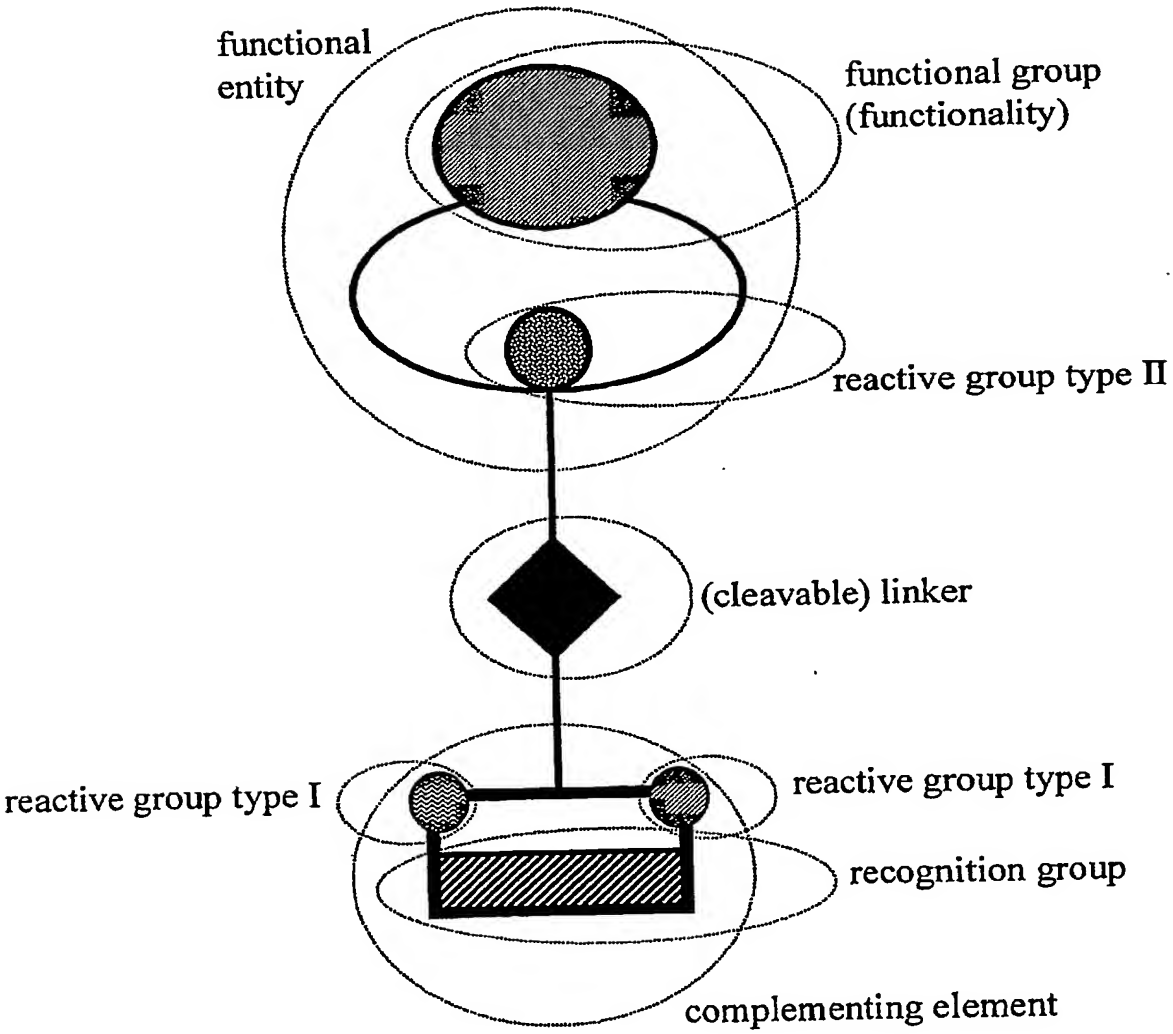


Figure 5. Building blocks and the polymers resulting from template directed incorporation of the building blocks and their polymerization and activation

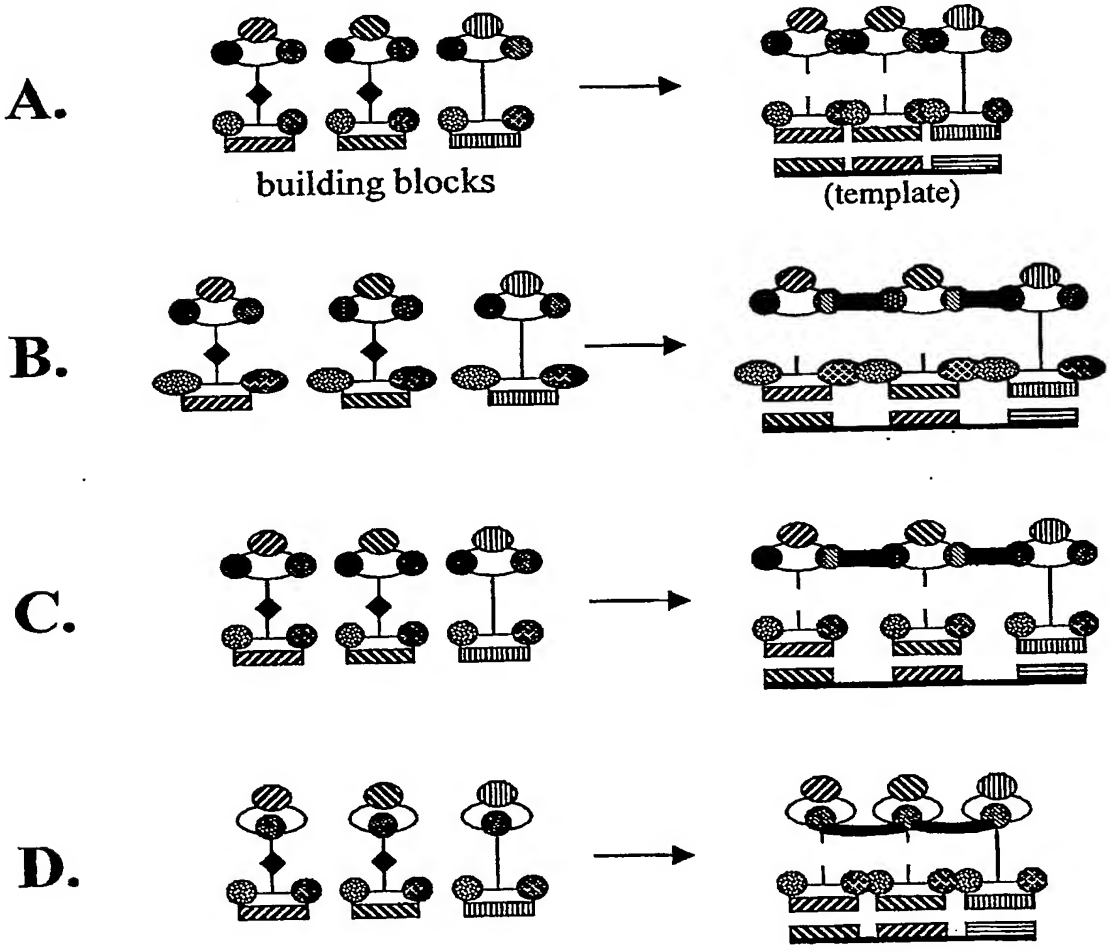


Figure 6. A derivatized nucleotide as building block.

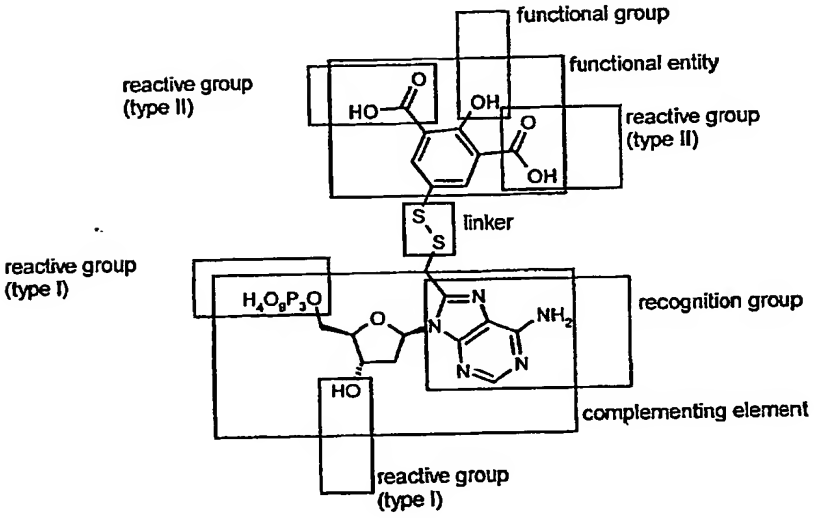


Figure 7. C-terminal tagging of a β-peptide - incorporation, polymerization and activation. an example

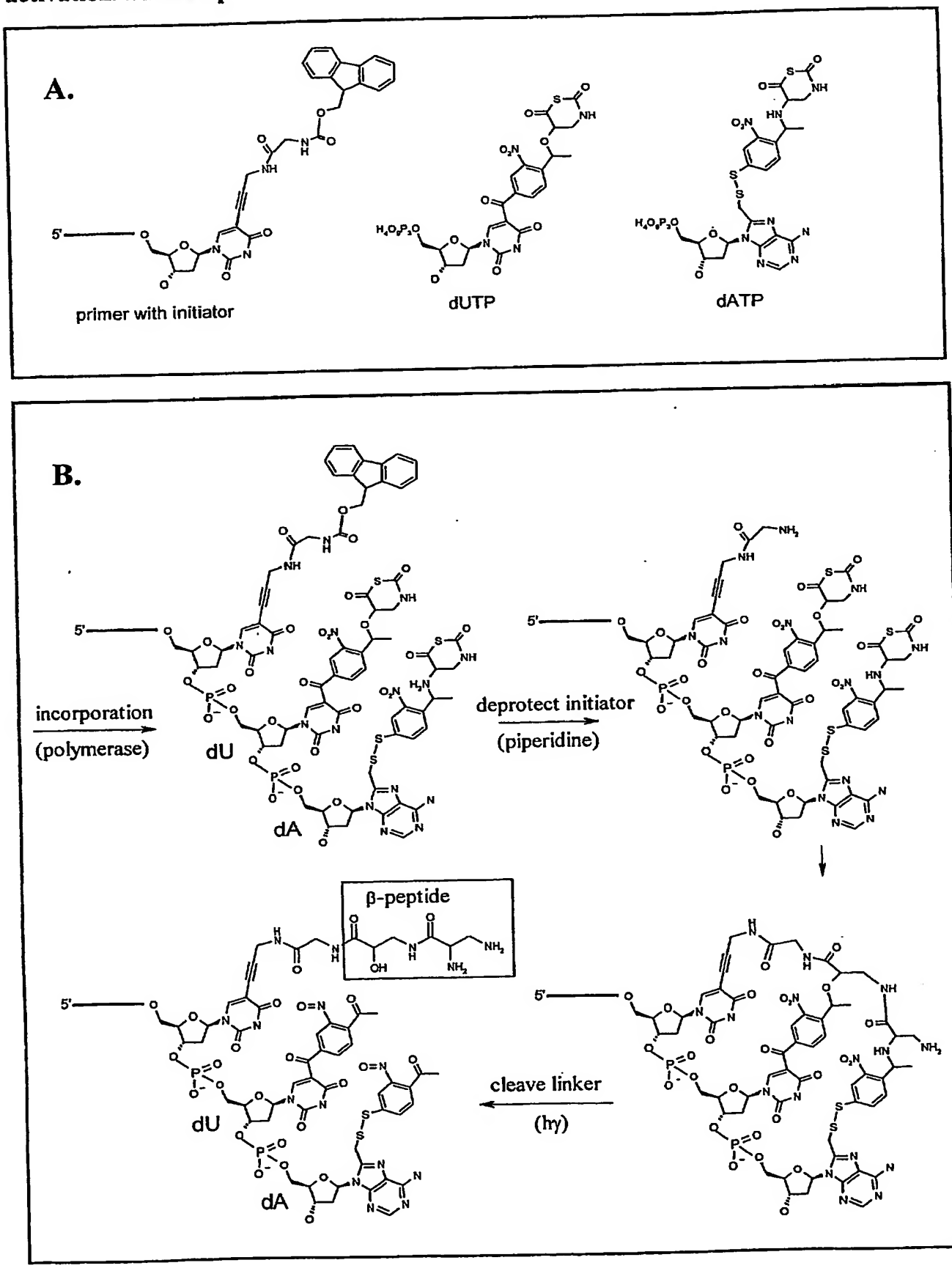


Figure 7. C-terminal tagging of a β-dipeptide - incorporation, polymerization and activation.

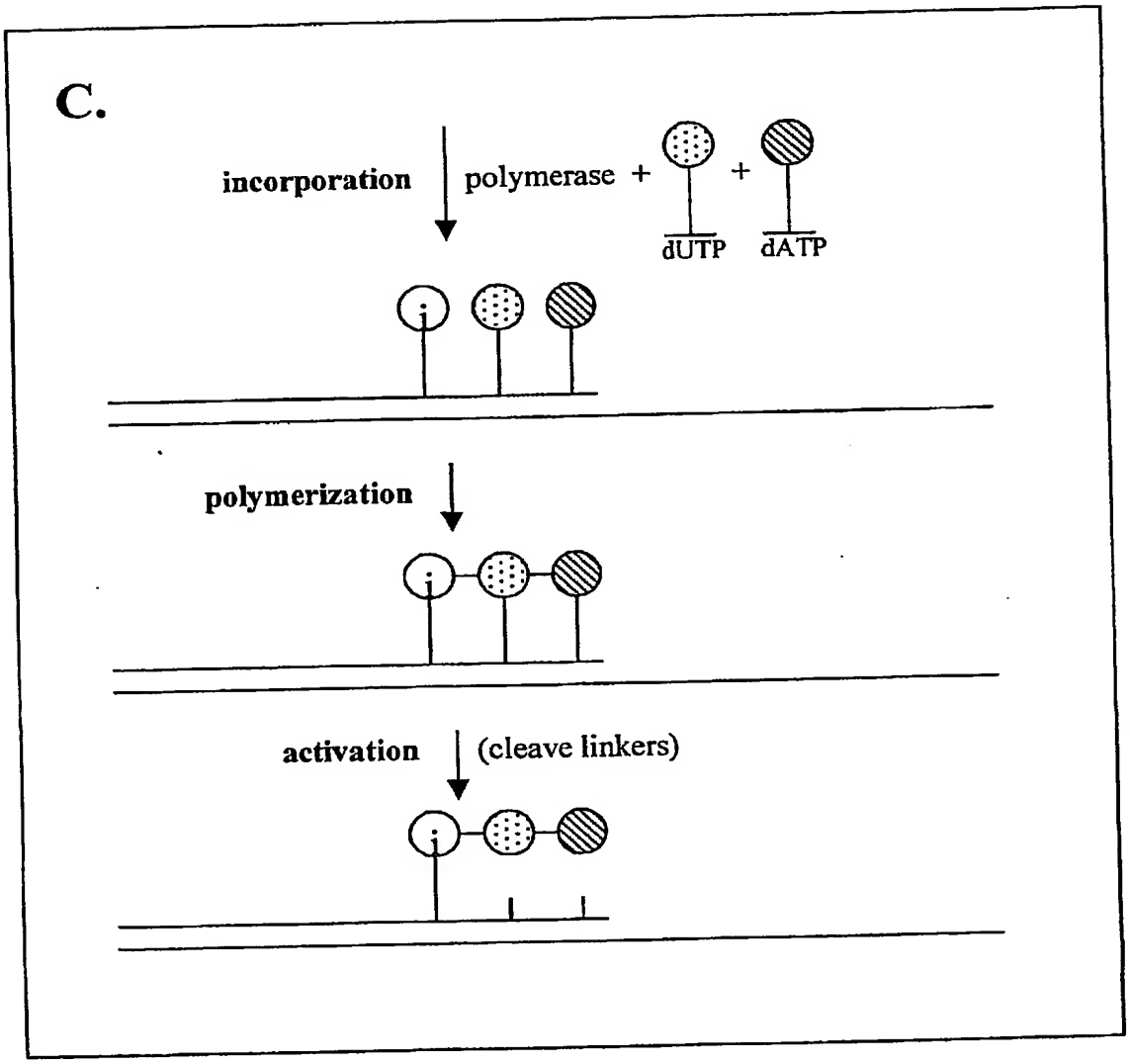


Figure 8. N-teminal tagging of a β-dipeptide - incorporation, polymerization and activation.

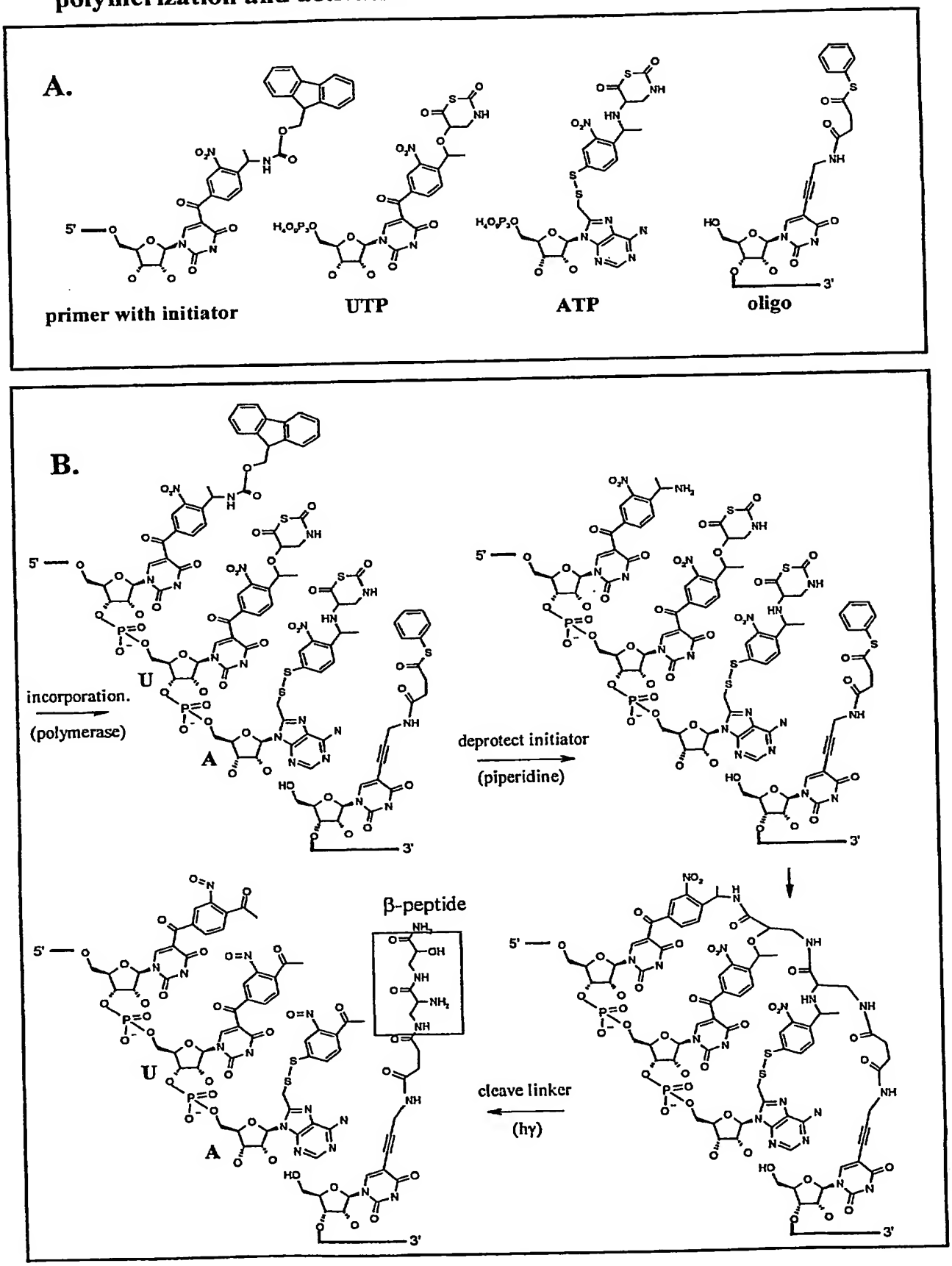


Figure 8. N-teminal tagging of a β-dipeptide - incorporation, polymerization and activation.

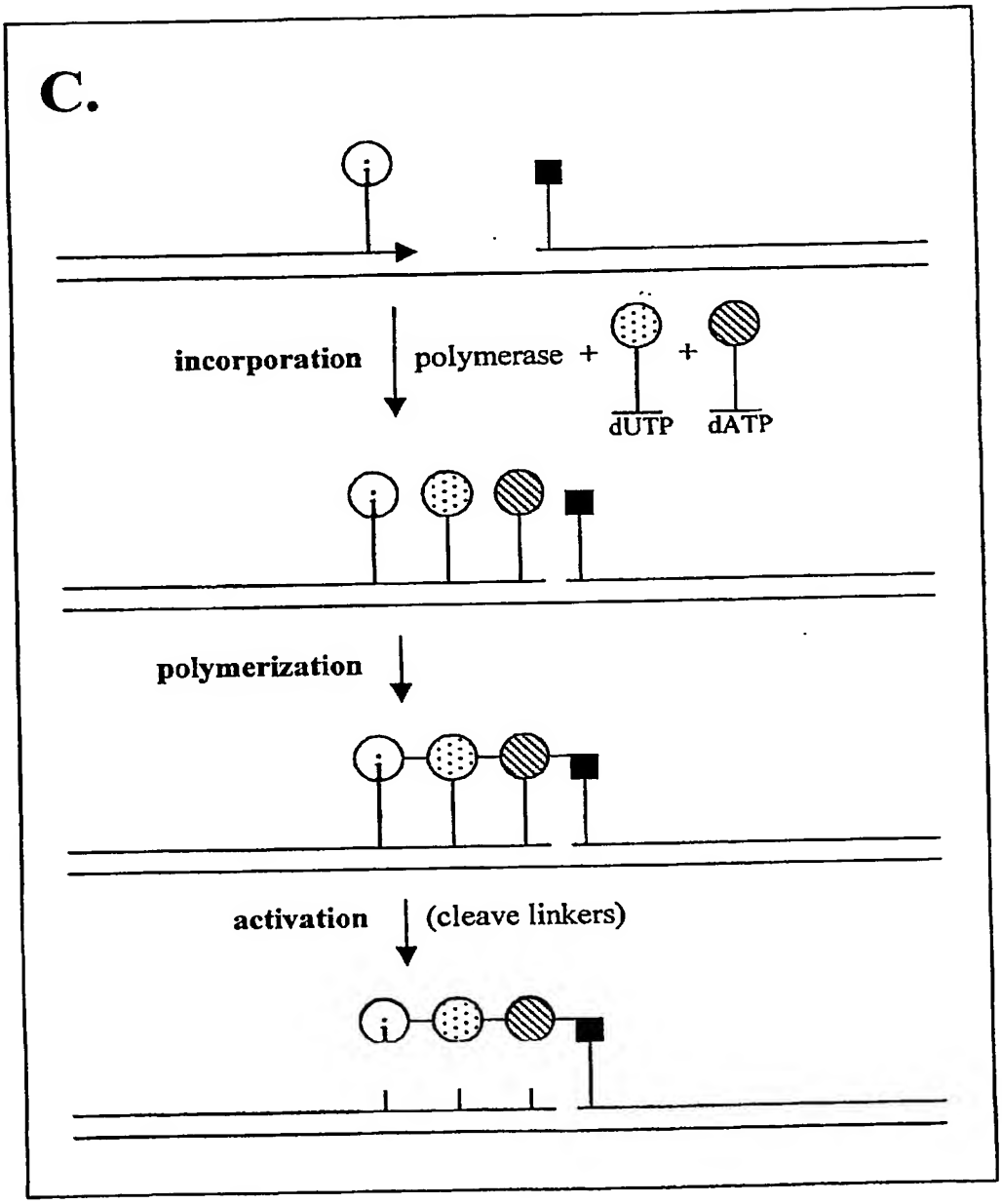


Figure 9. Nucleotide-derivatives that are known to be incorporated by polymerases

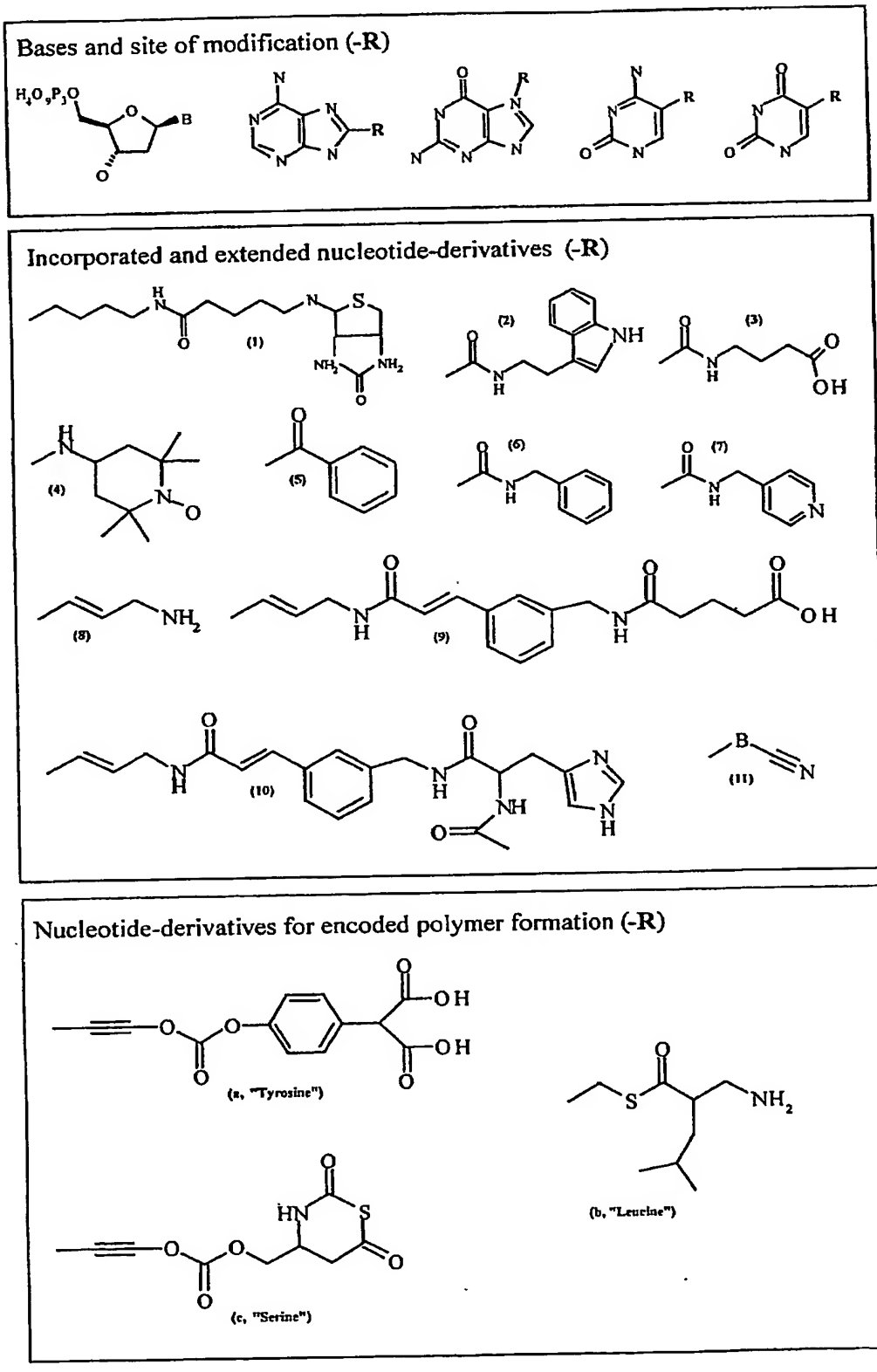


Figure 10. Cleavable linkers and protection groups, cleaving agents and cleavage products.

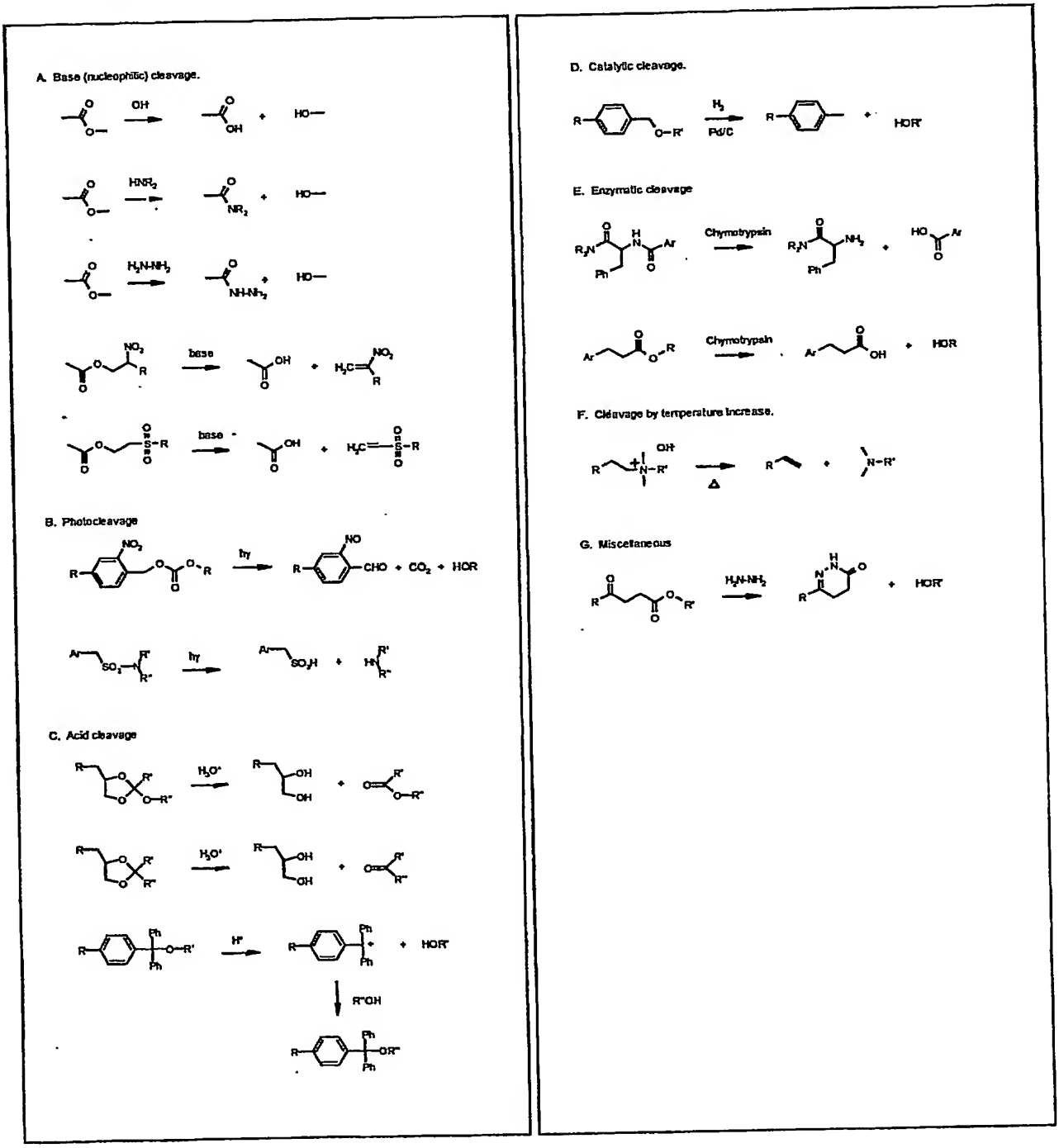


Figure 11. Polymerization by reaction between neighboring reactive groups type II.

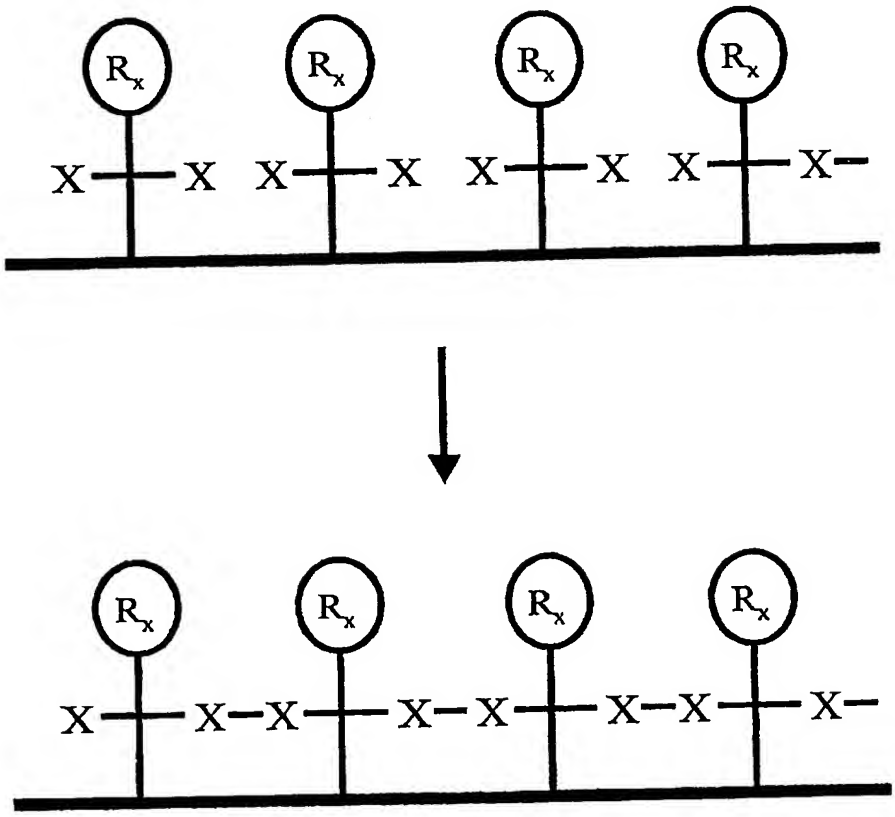


Figure 11, ex.1. Coumarin-based polymerization.

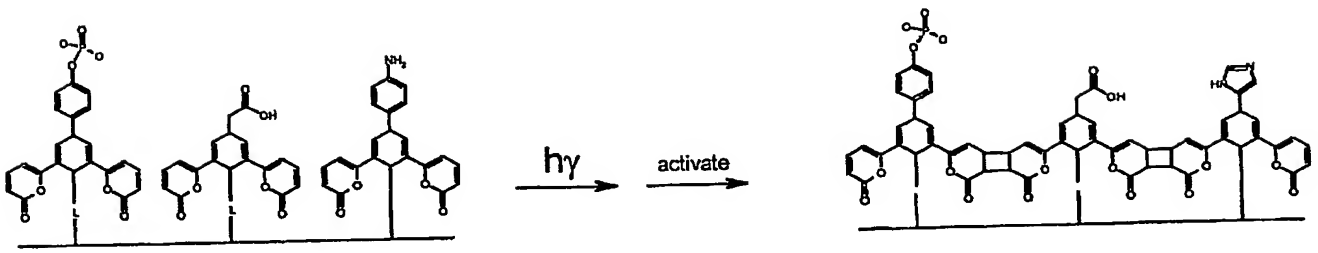


Figure 12. Polymerization between neighboring non-identical reactive groups type II.

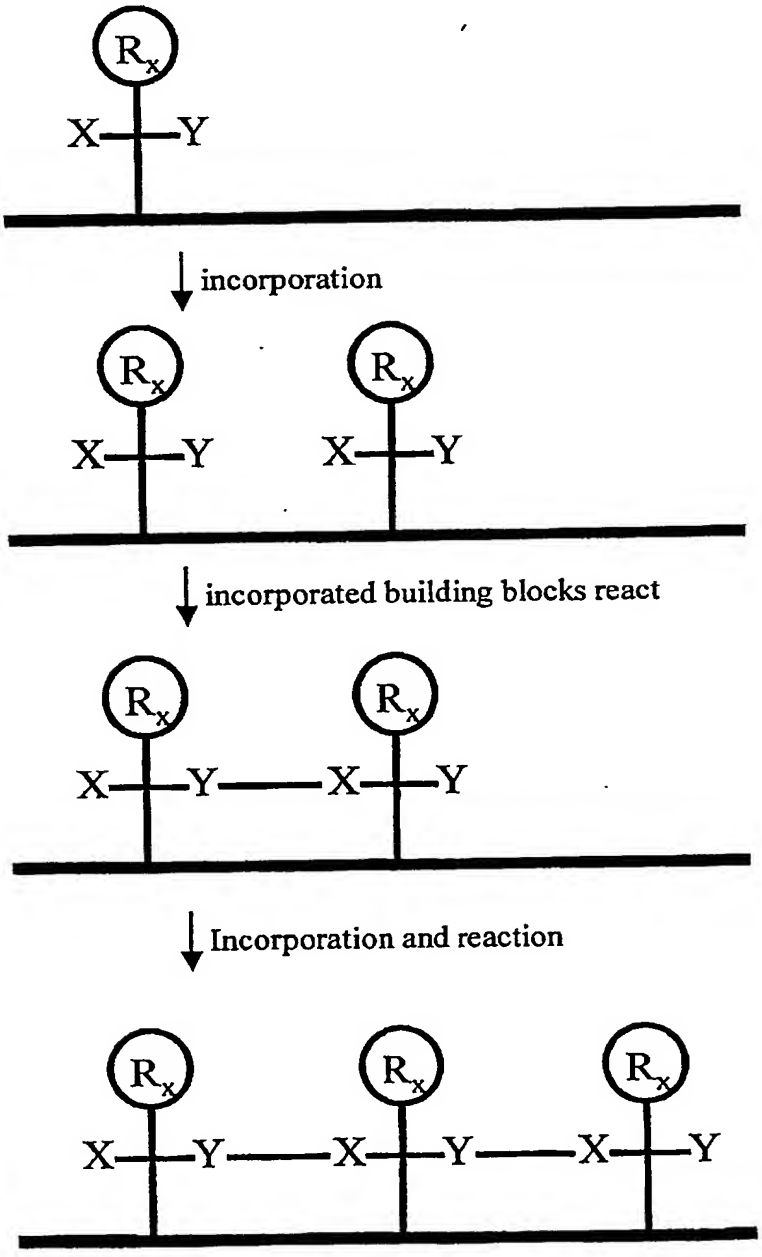


Figure 13. Cluster formation in the absence of directional polymerization.

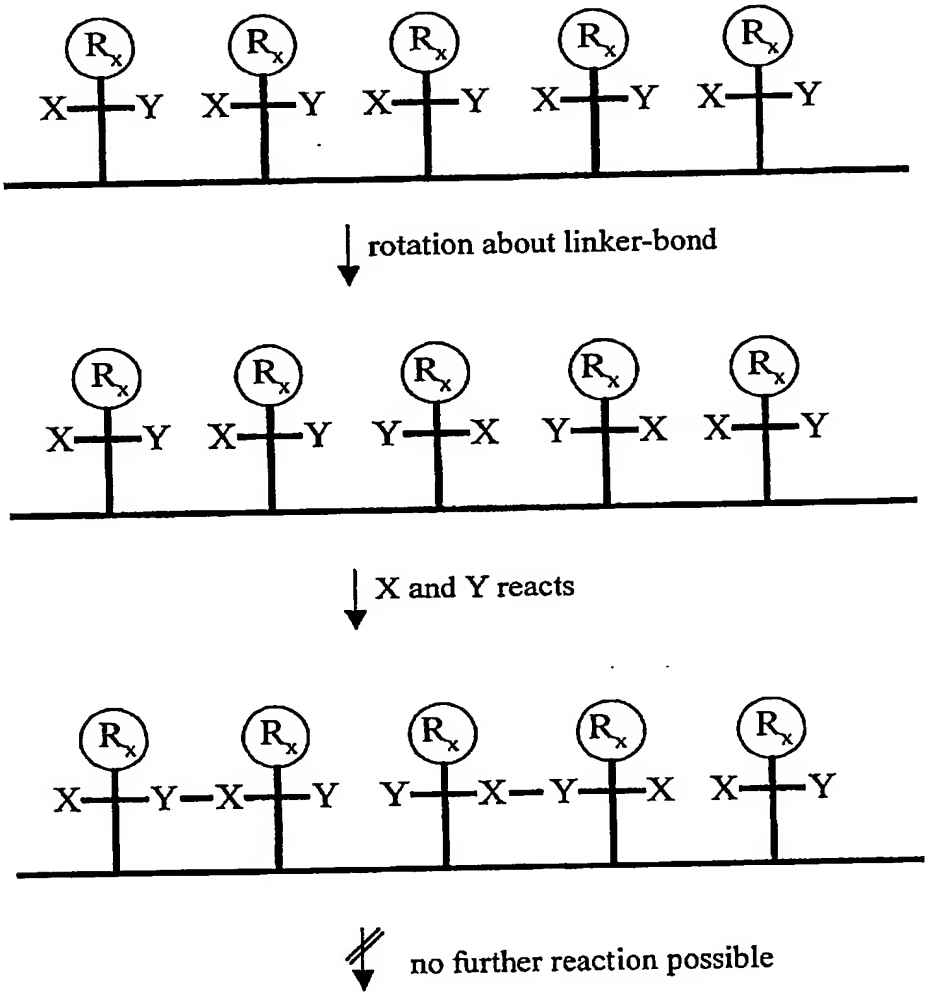


Figure 14. Zipping-polymerization and simultaneous activation.

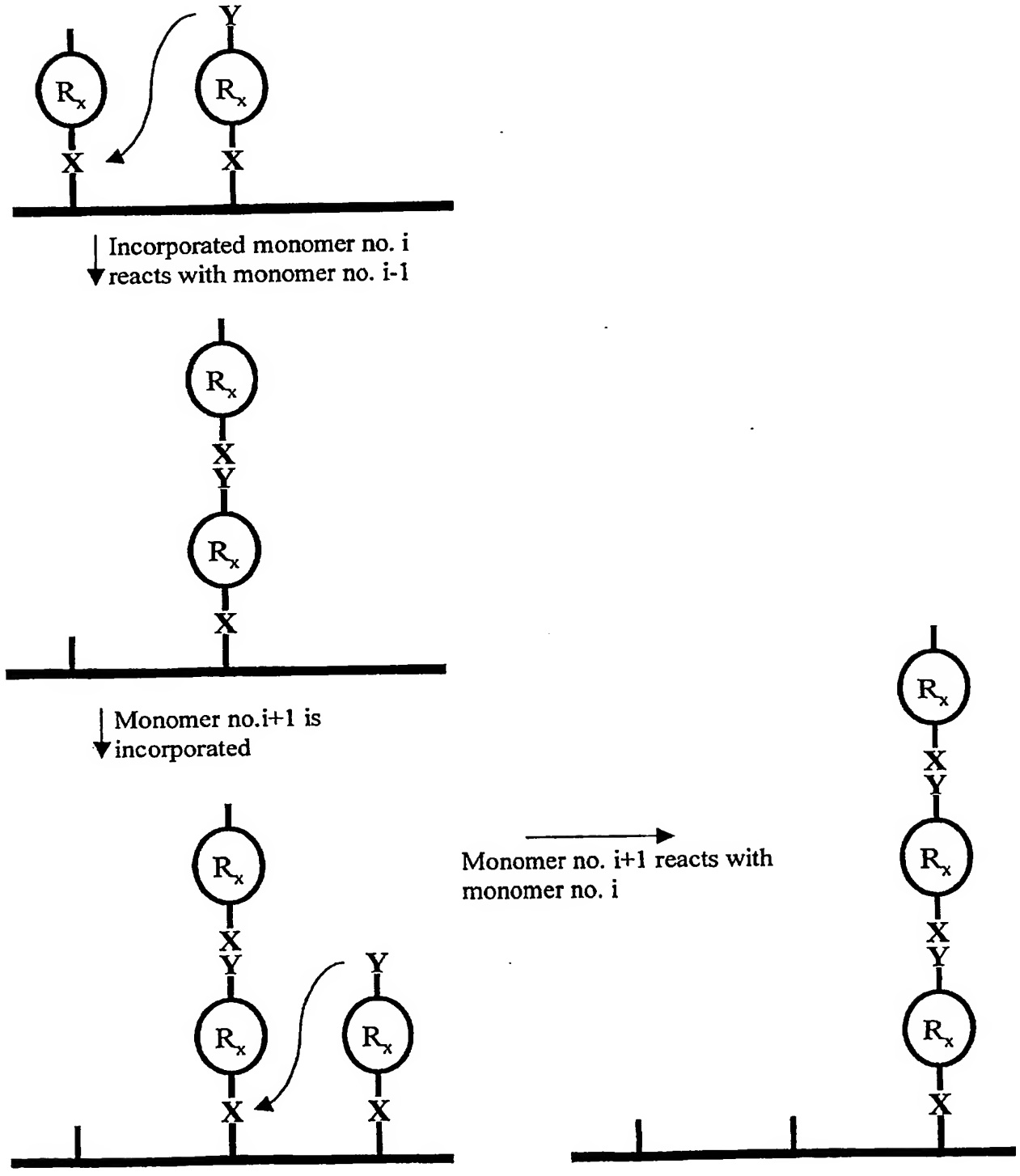


Figure 14, example 1. Polymerization and activation (thioesters)

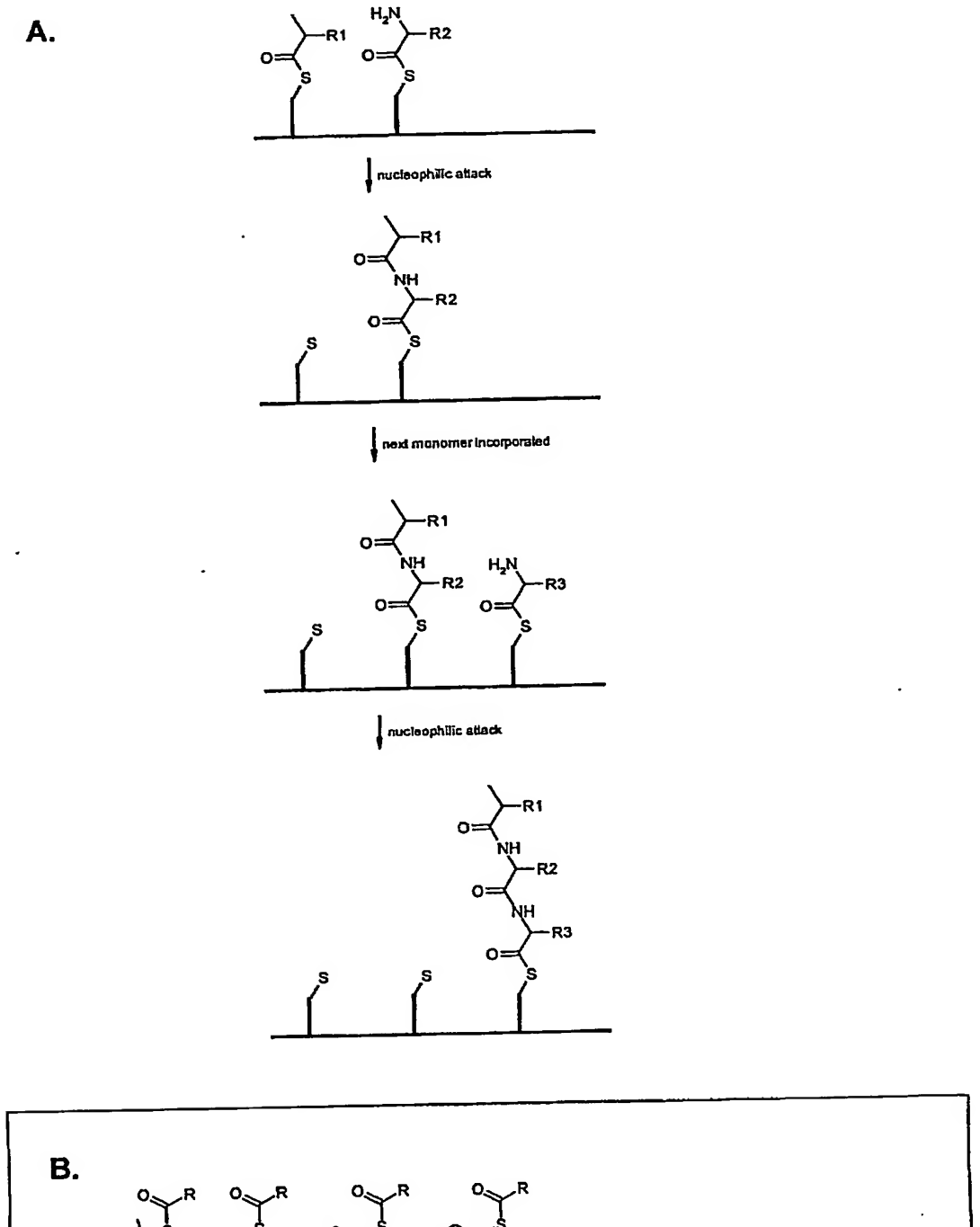


Figure 14, example 2. Polyamine formation and activation

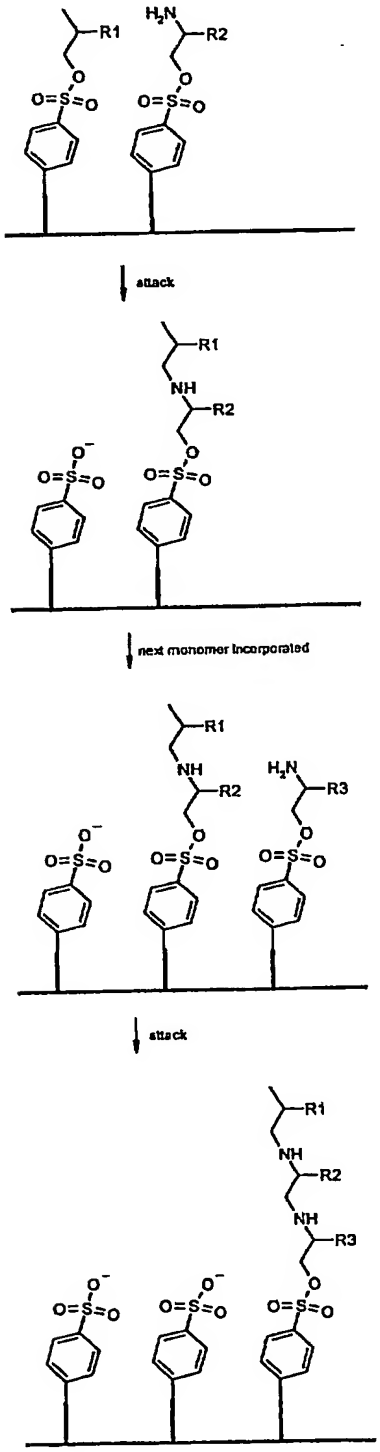


Figure 15. "Fill-in" polymerization (symmetric XX monomers).

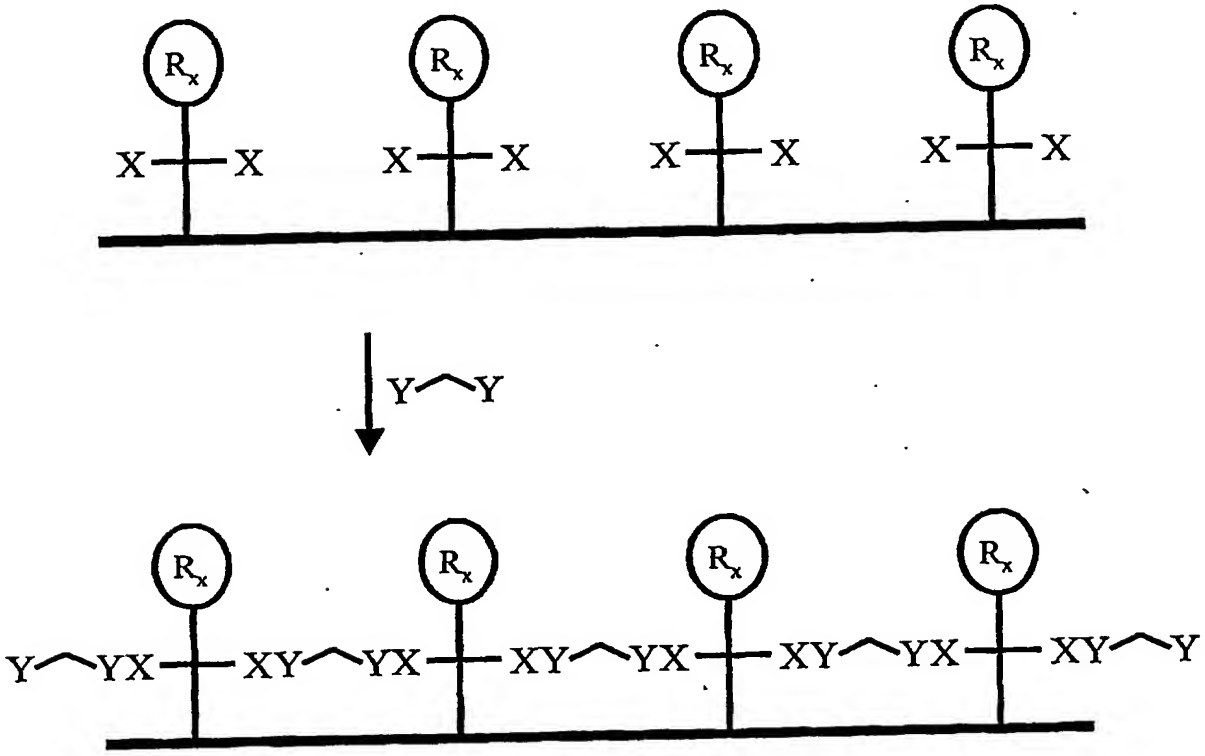


Figure 15, example 1. Poly-imine formation by fill-in polymerization.

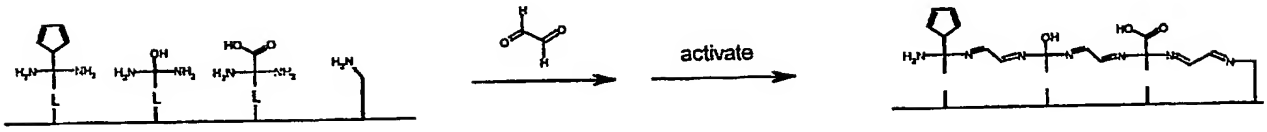


Figure 15, example 2. Polyamide formation.

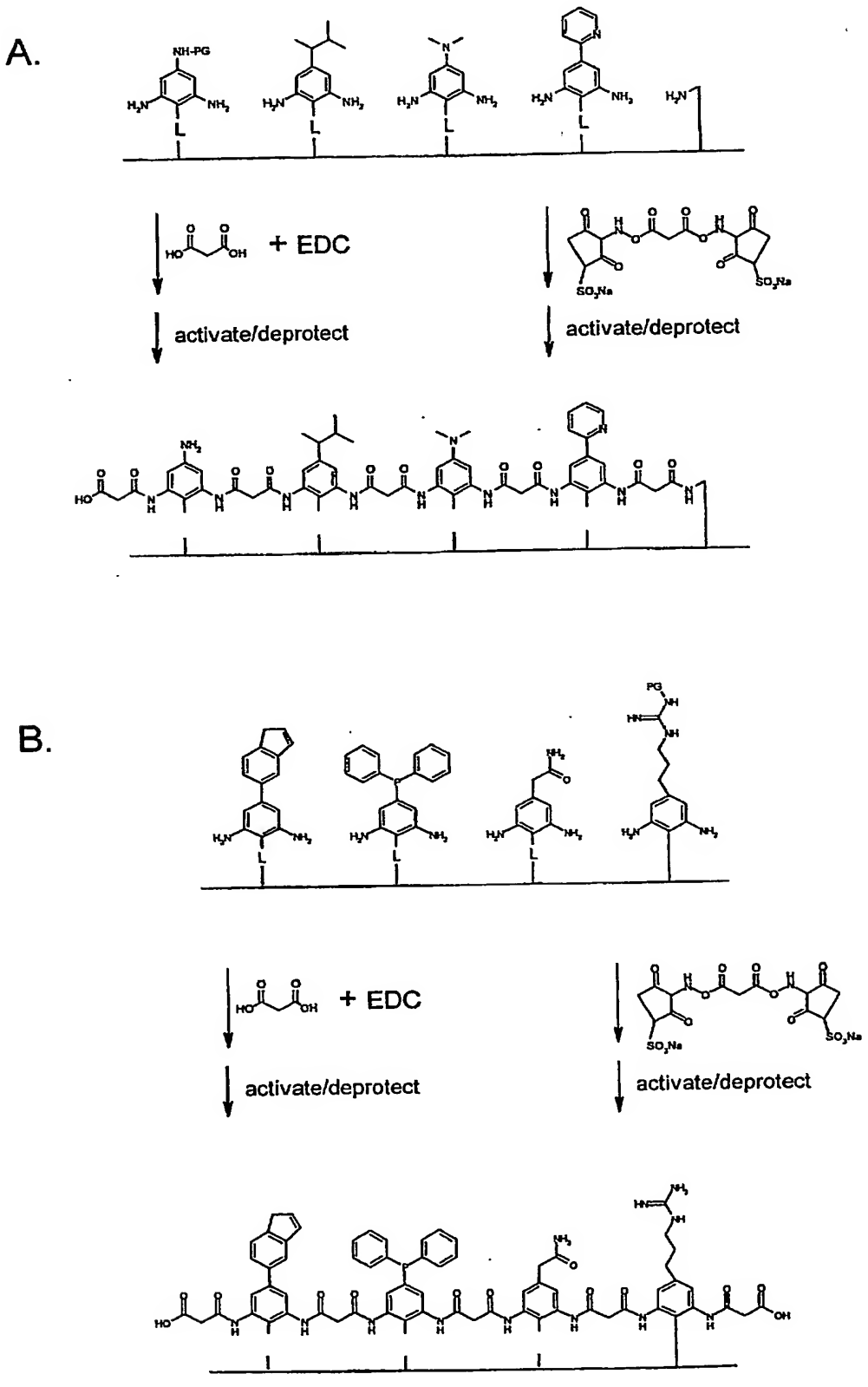


Figure 15, example 3. Polyurea formation

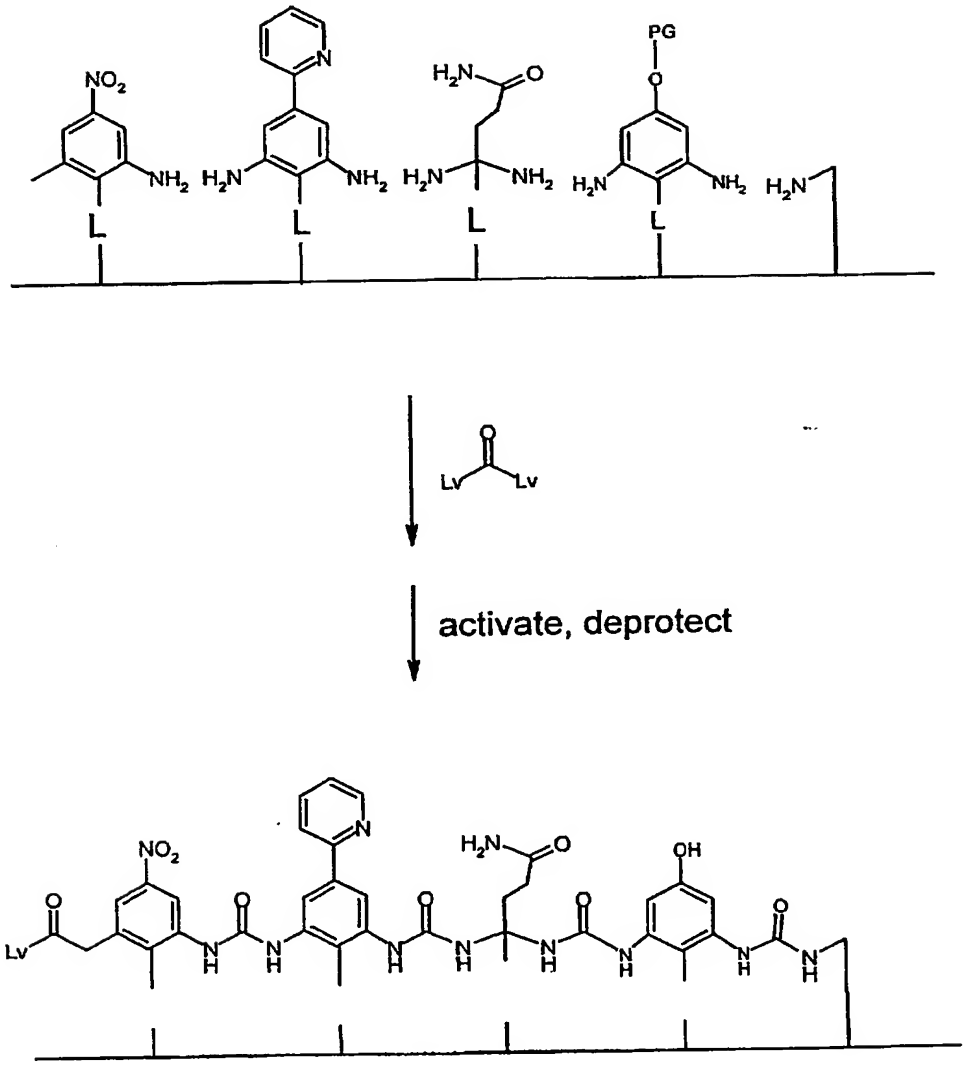


Figure 15, example 4. Chiral and achiral polyamide backbone formation

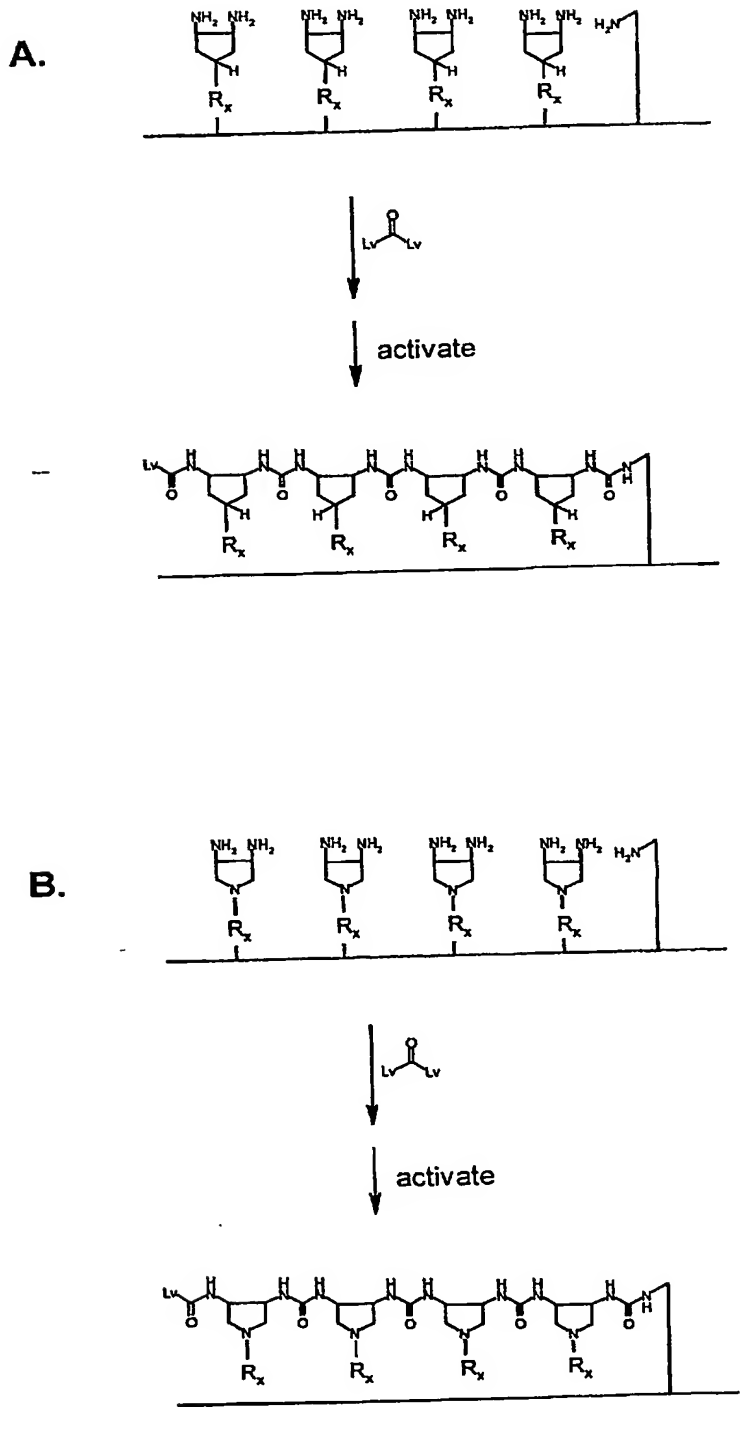


Figure 15, example 5. Polyphosphodiester formation.

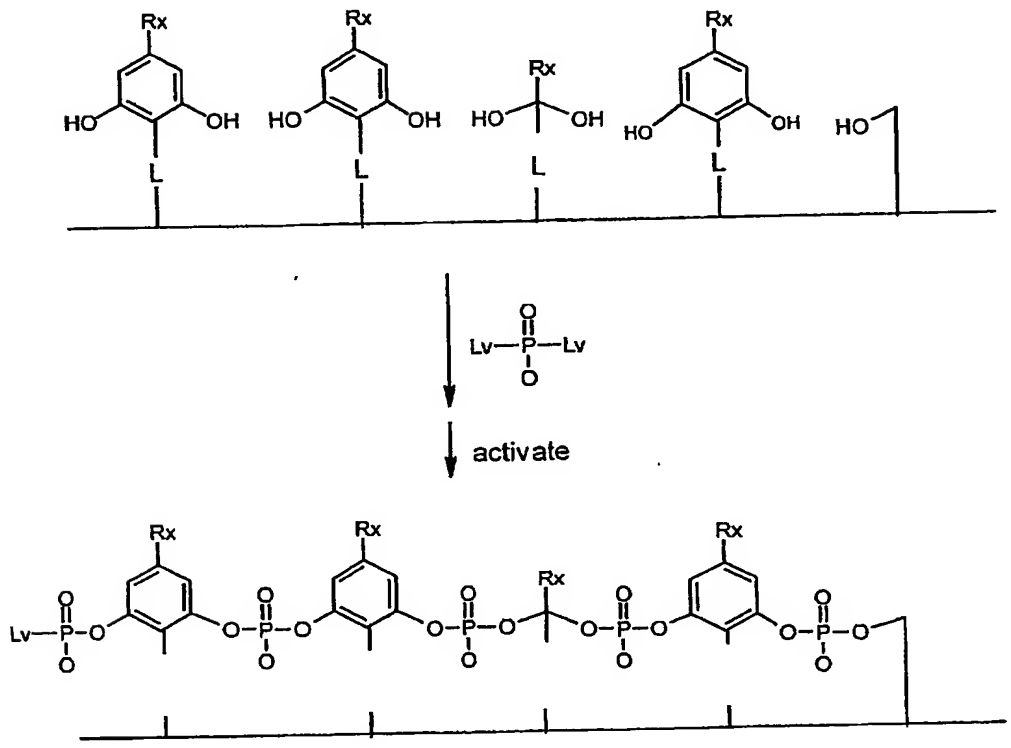


Figure 15, example 6. Polyphosphodiester formation with one reactive group in each monomer building

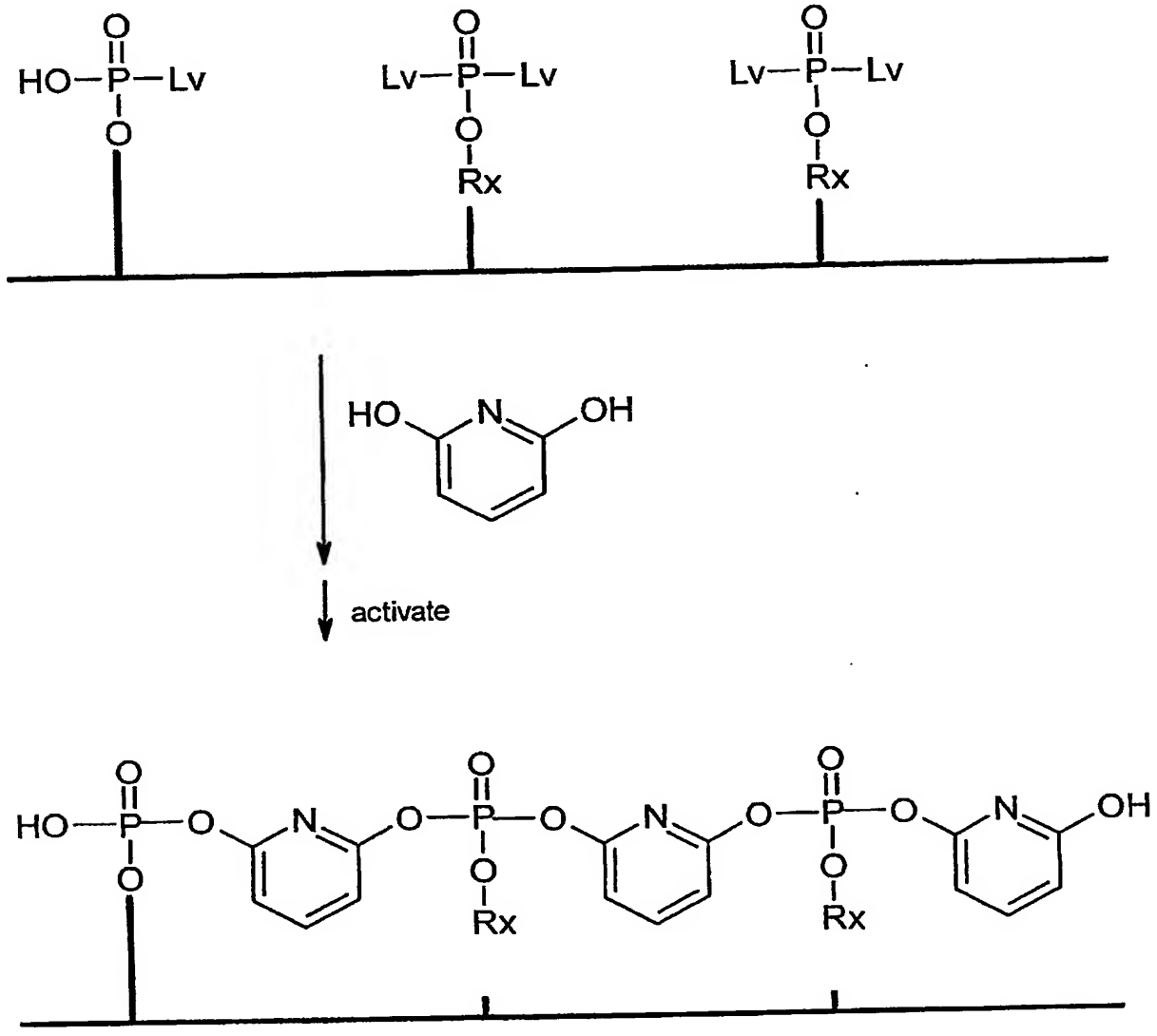


Figure 15, example 7. Pericyclic, "fill-in" polymerization.

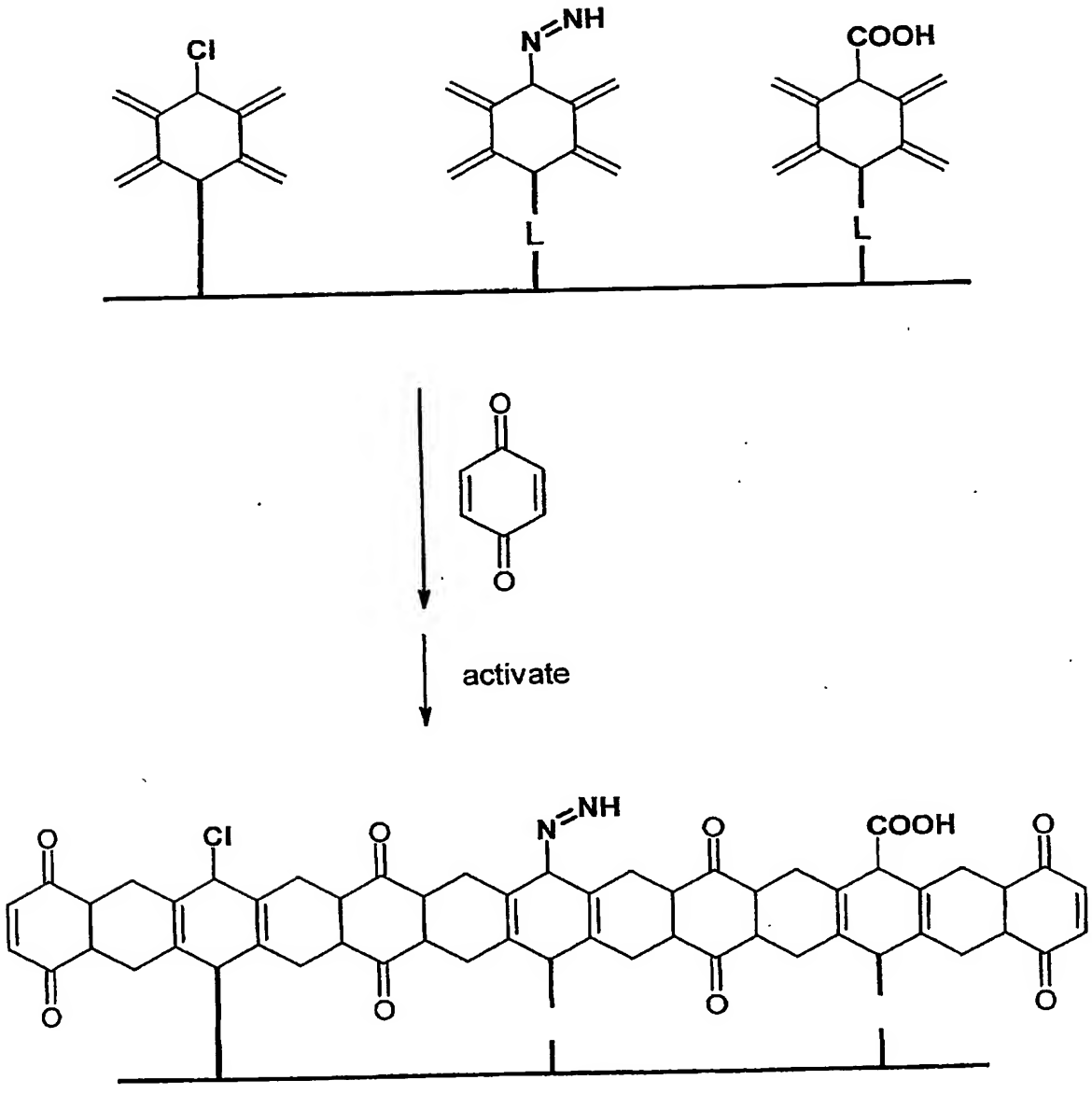


Figure 16. "Fill-in" polymerization (asymmetric XS monomers).

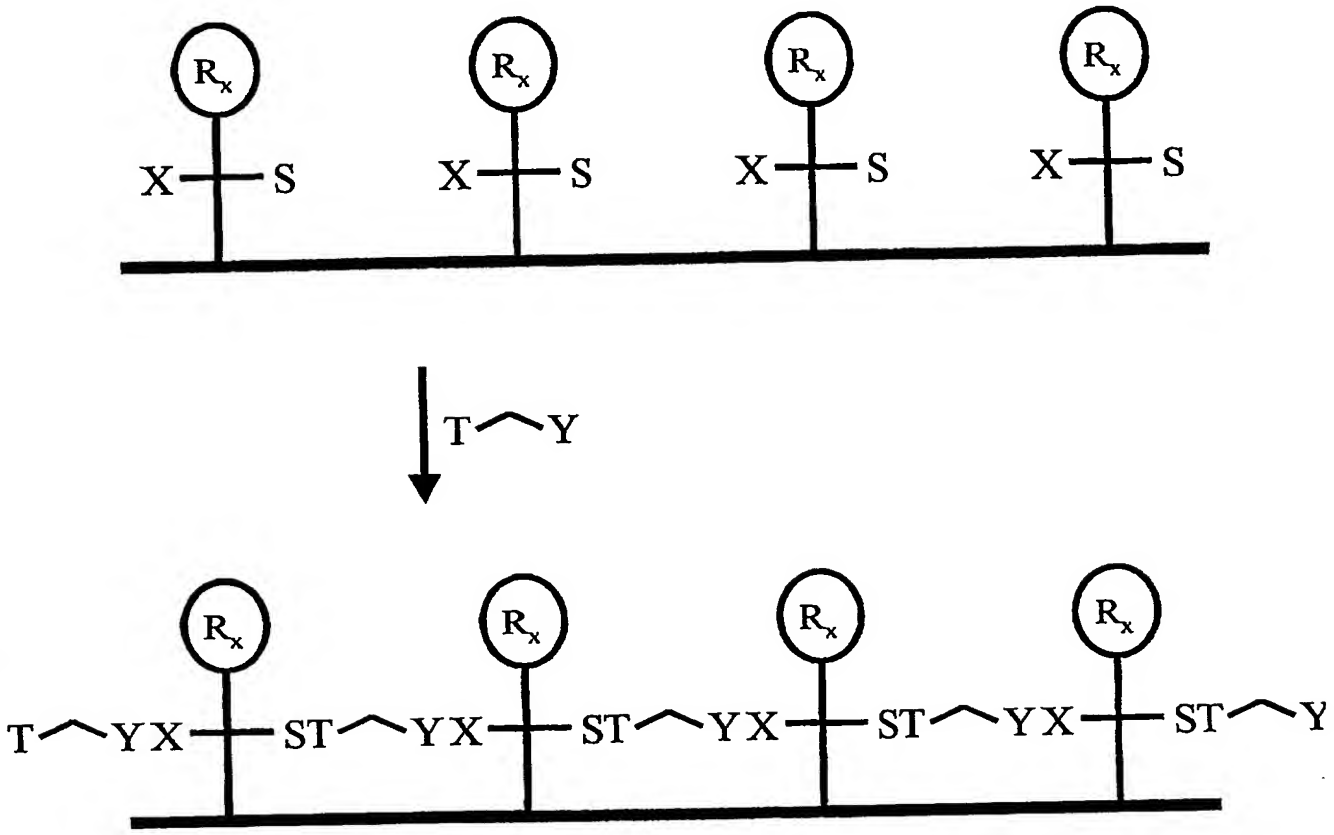


Figure 16, example 1. Fill-in polymerization by ketone-hydrazide reaction and by modified Staudinger ligation

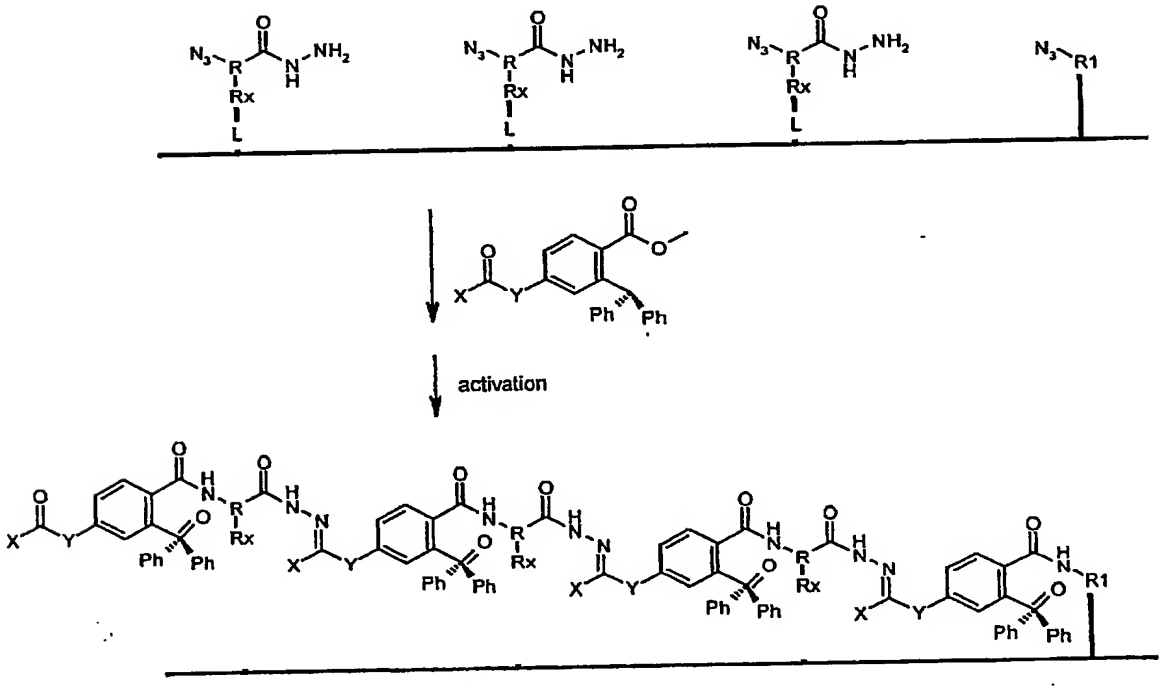


Figure 17. "Zipping" polymerization.

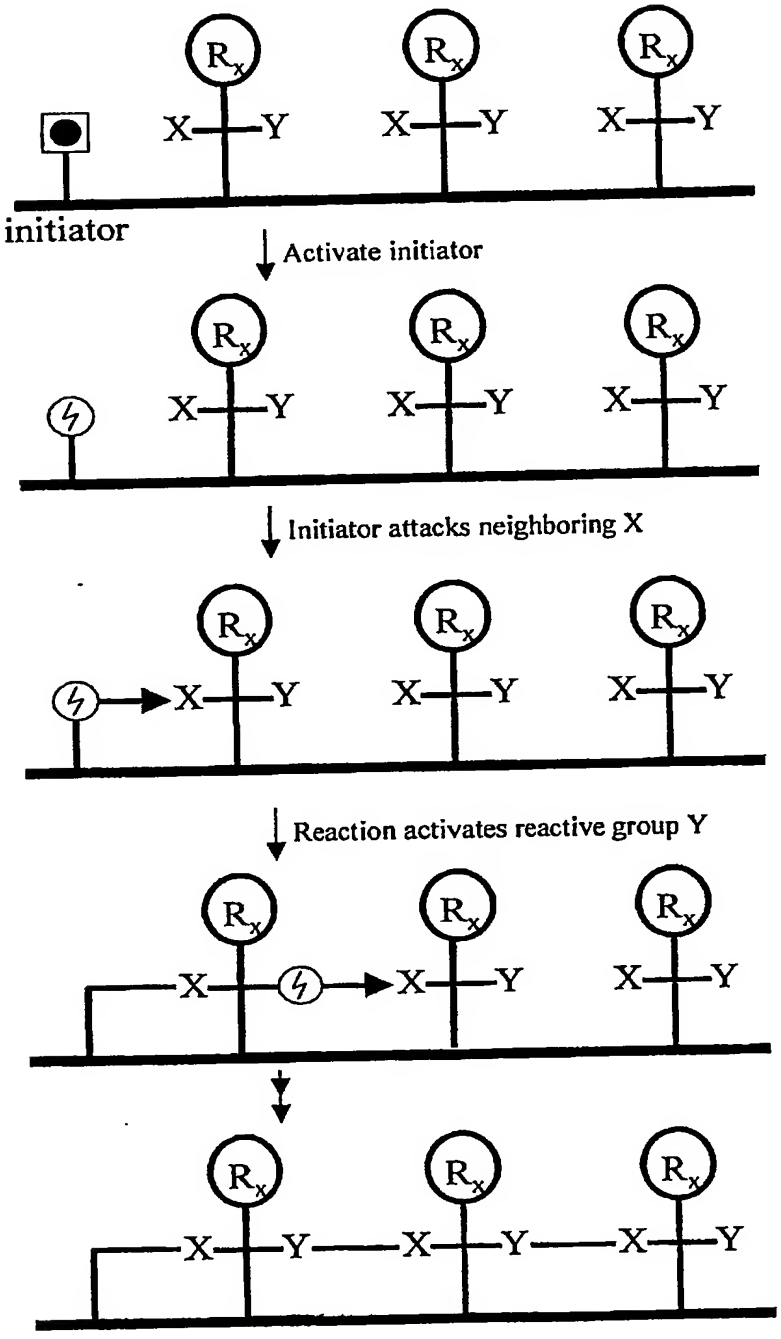


Figure 17, example 1. Radical polymerization

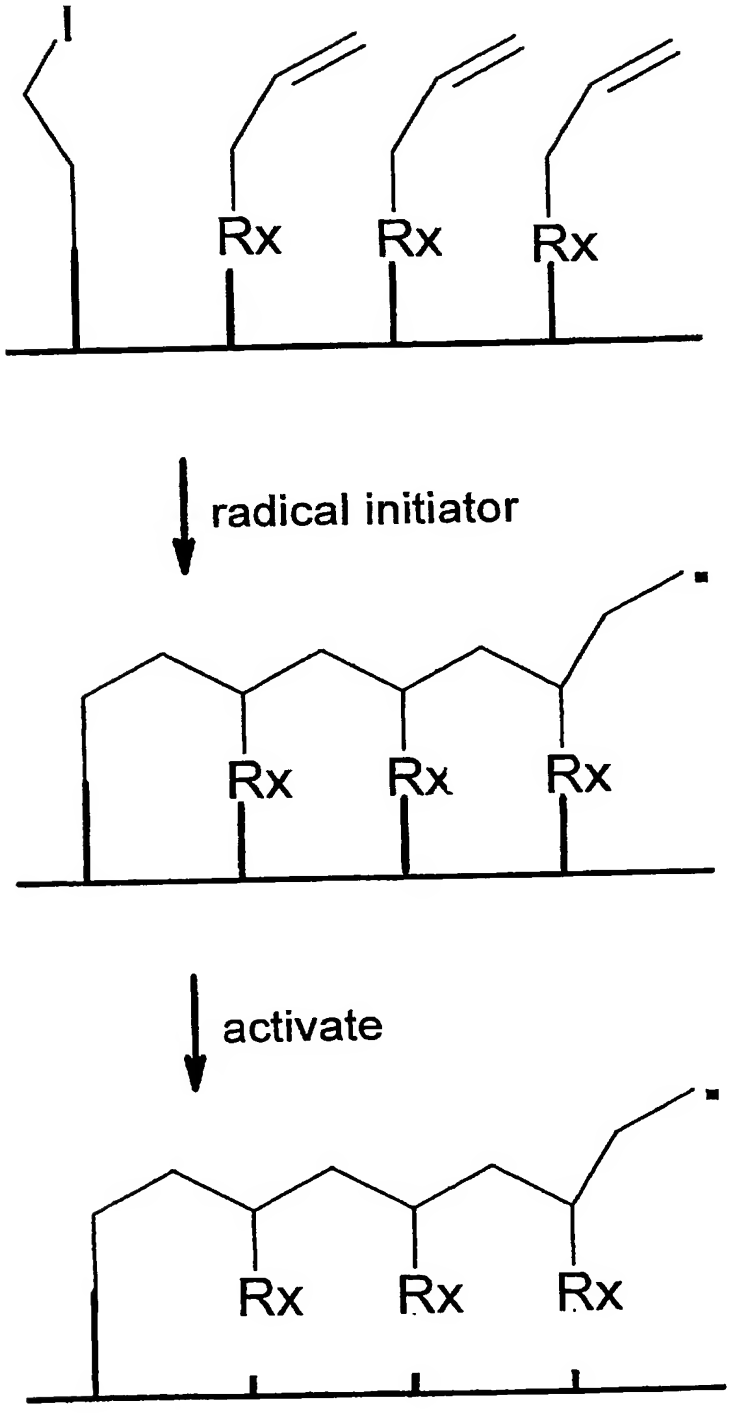


Figure 17, example 2. Cationic polymerization

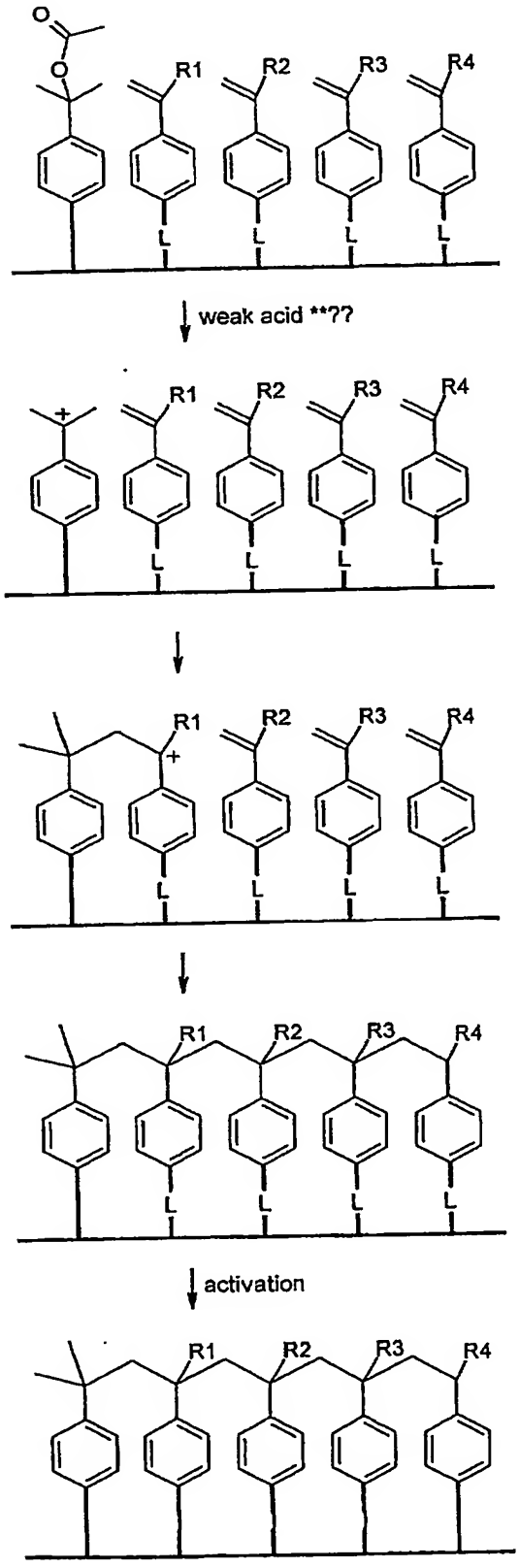


Figure 18. Zipping polymerization by ring opening.

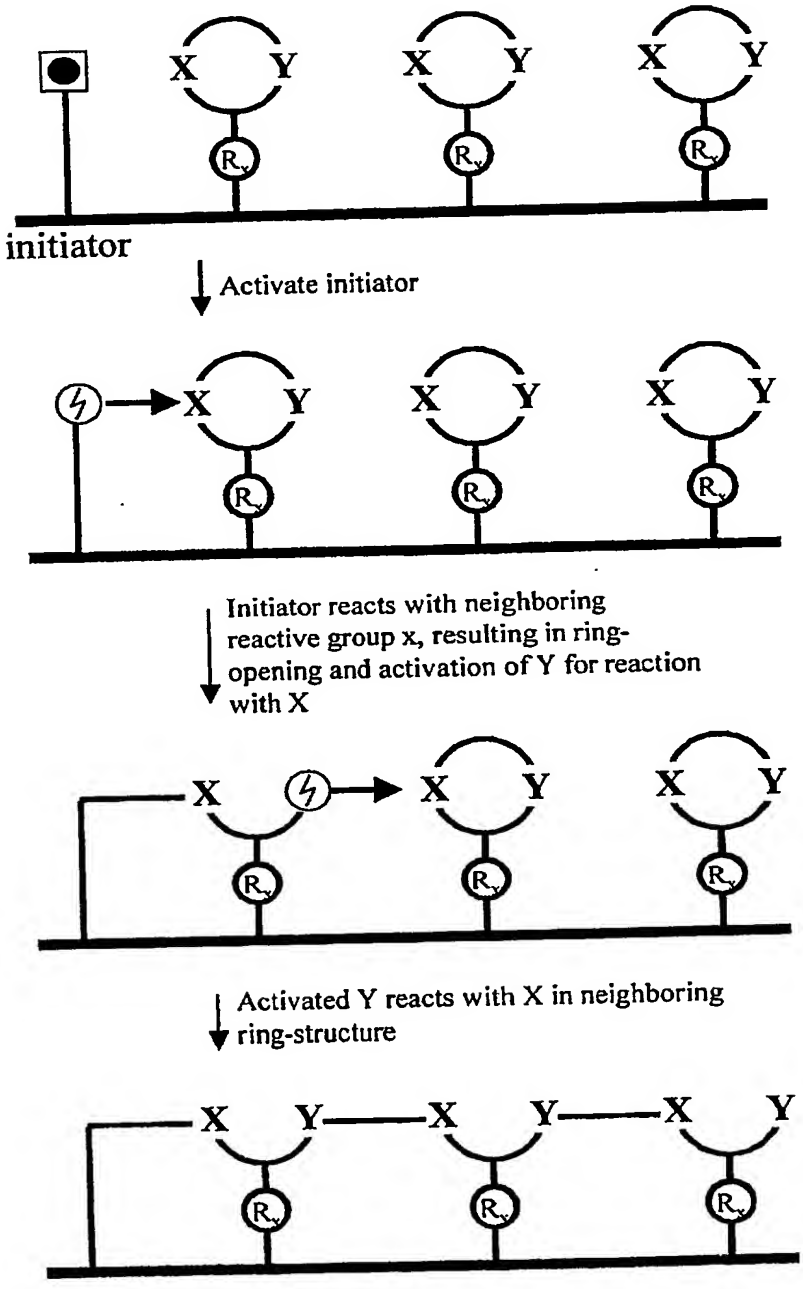


Figure 18, example 1. "Zipping" polymerization of N-thiocarboxyanhydrides, to form β-peptides.

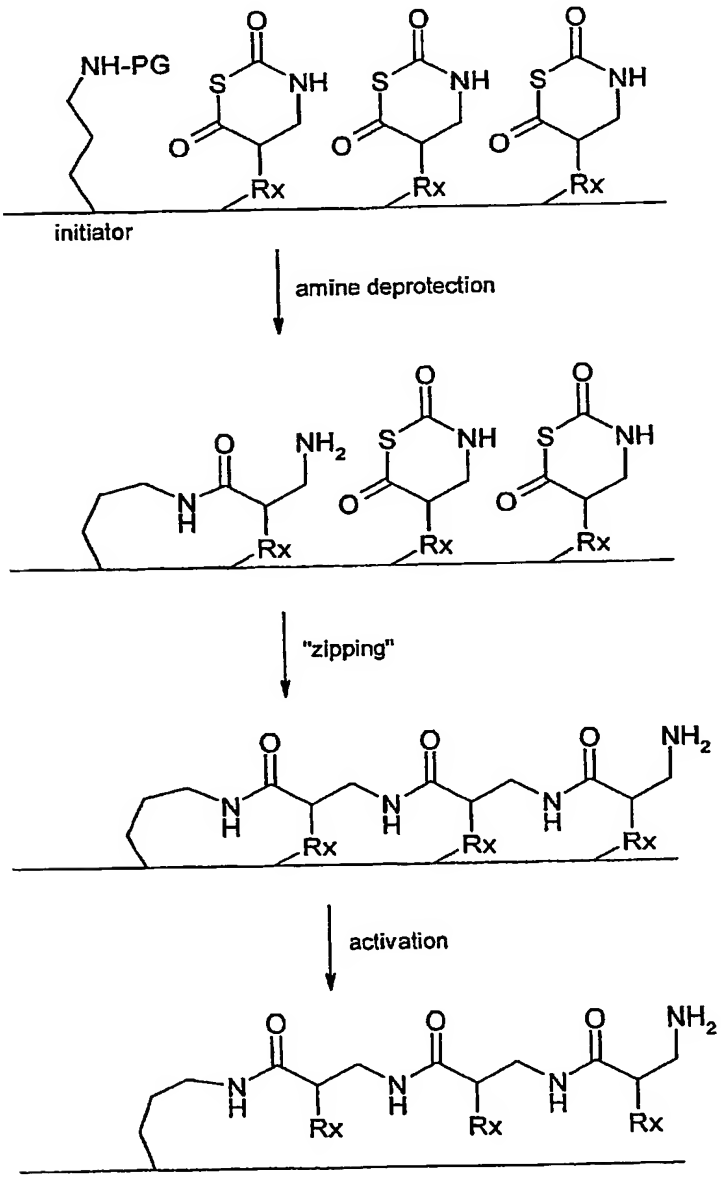


Figure 18, example 2. "Zipping" polymerization of 2,2-diphenylthiazinanone units to form β-peptides.

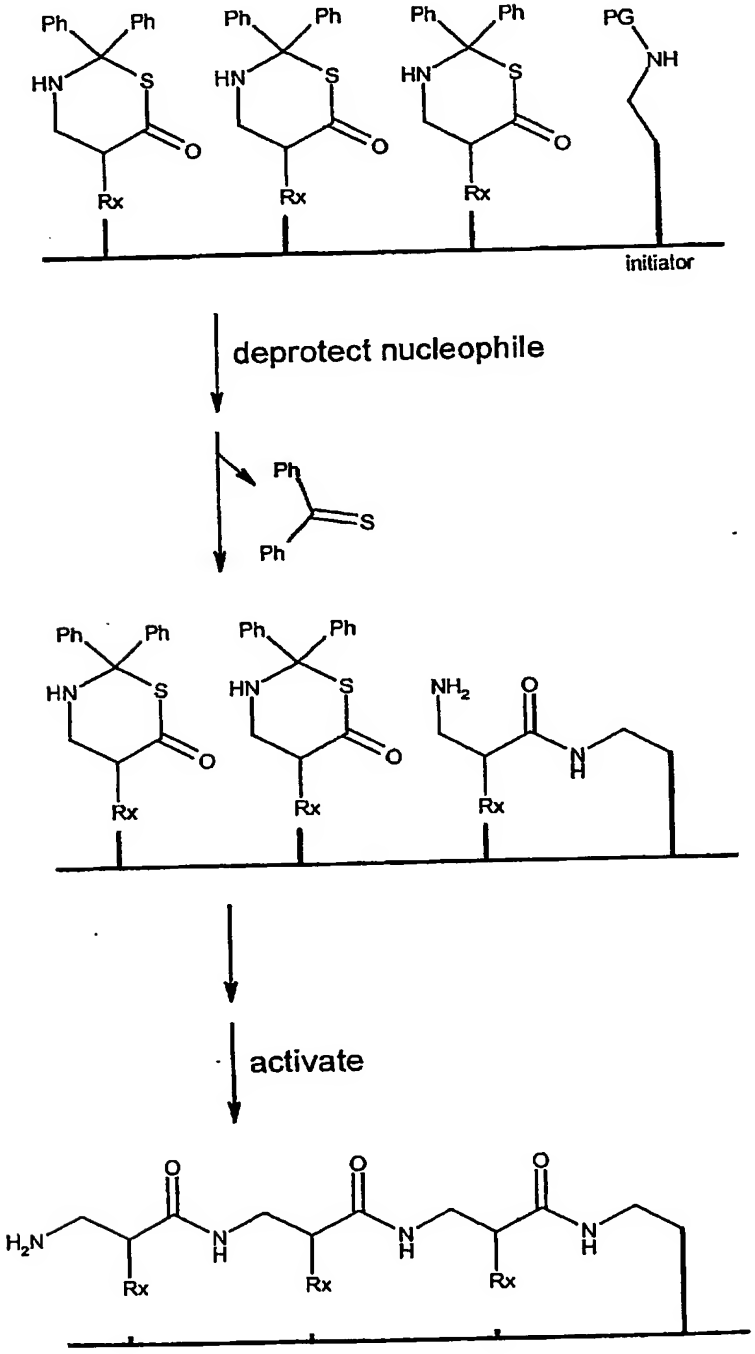


Figure 18, example 3. Polyether formation by ring-opening polymerization

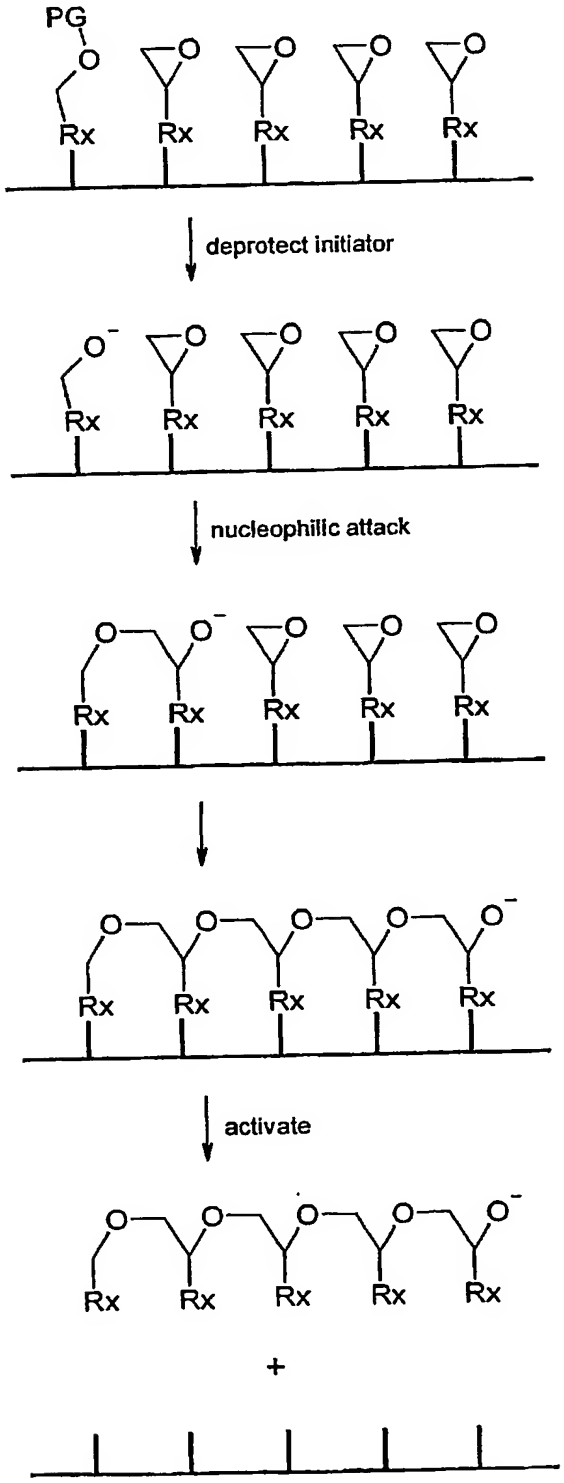


Figure 19. Zipping-polymerization and activation by rearrangement.

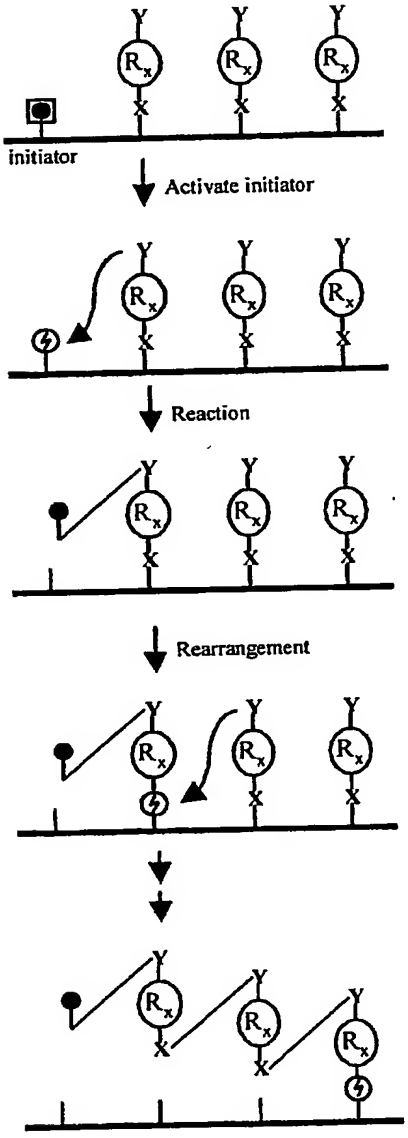


Figure 20. Zipping-polymerization and activation by ring opening.

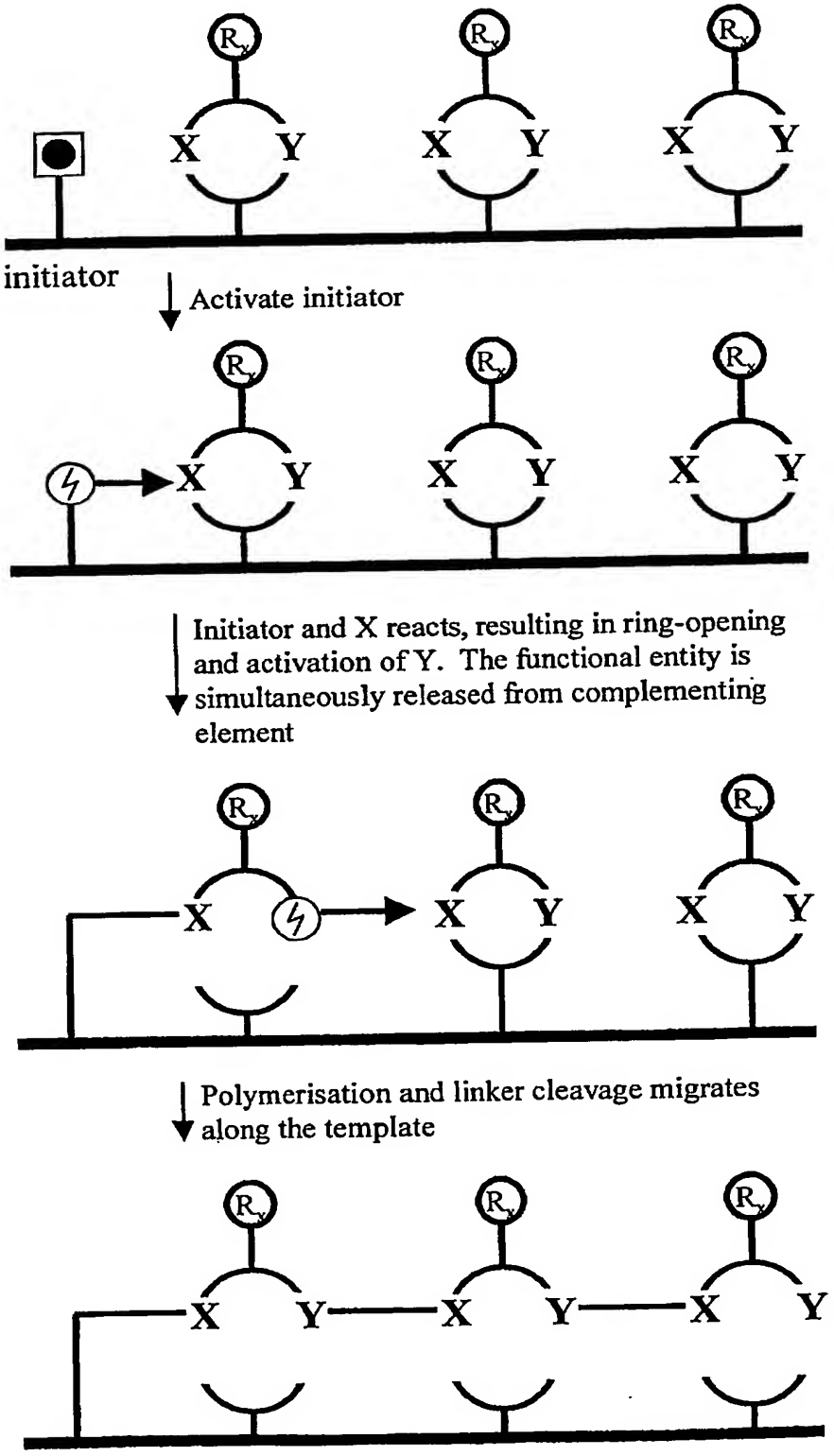
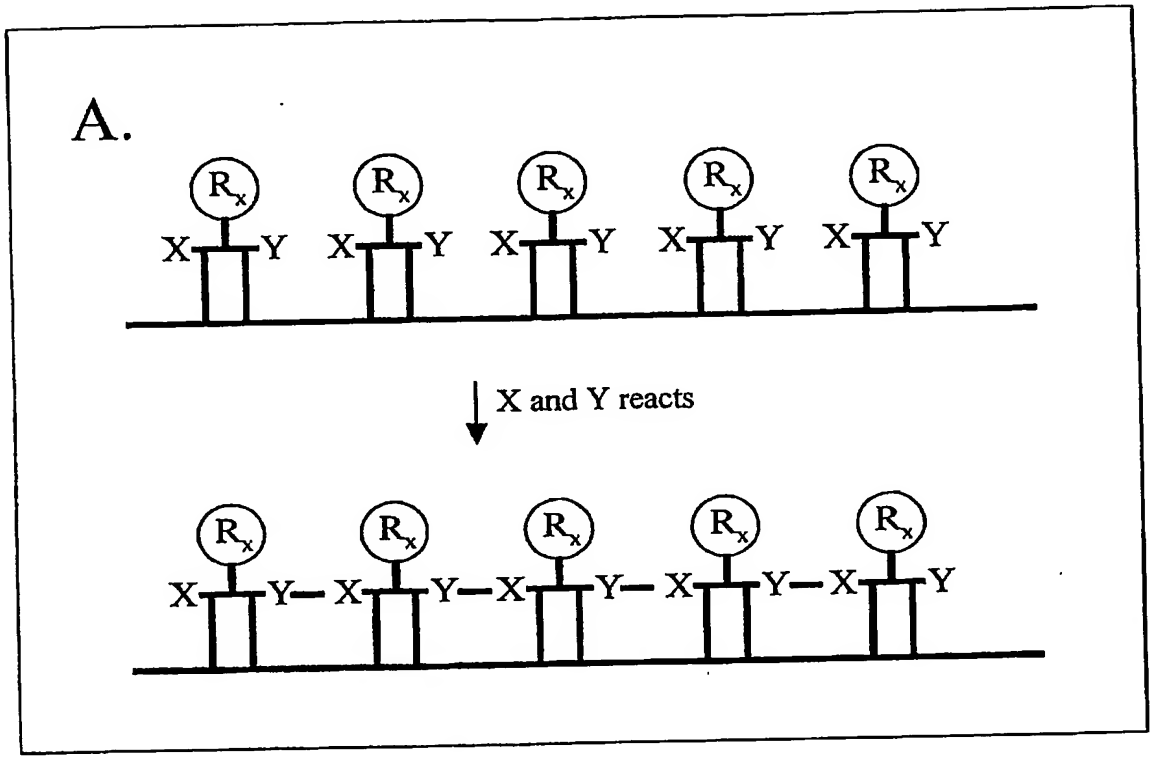


Figure 21. Directional polymer formation using fixed functional units.



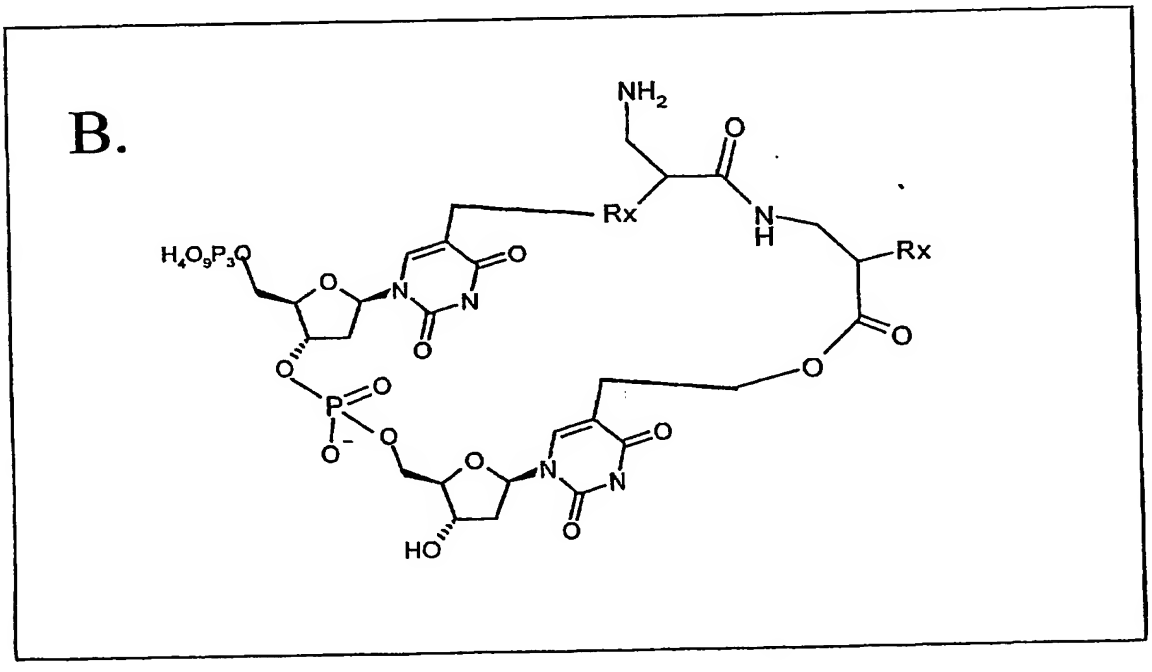


Figure 22. Templated polymers.

- alpha-, beta-, gamma-, and omega-peptides
- mono-, di- and tri-substituted peptides
- L- and D-form peptides
- cyclohexane- and cyclopentane-backbone modified beta-peptides
- vinylogous polypeptides
- glycopolypeptides
- polyamides
- vinylogous sulfonamide peptide
- Polysulfonamide
- conjugated peptide (i.e., having prosthetic groups)
- Polyesters
- Polysaccharides
- Polycarbamates
- Polycarbonates
- Polyureas
- poly-peptidylphosphonates
- Azatides
- peptoids (oligo N-substituted glycines)
- Polyethers
- ethoxyformacetal oligomers
- poly-thioethers
- polyethylene glycols (PEG)
- Polyethylenes
- Polydisulfides
- polyarylene sulfides
- Polynucleotides
- PNAs
- LNAs
- Morpholinos
- oligo pyrrolinone
- polyoximes
- Polyimines
- Polyethyleneimine
- Polyacetates
- Polystyrenes
- Polyacetylene
- Polyvinyl
- Lipids
- Phospholipids
- Glycolipids
- polycycles (aliphatic)
- polycycles (aromatic)
- polyheterocycles
- Proteoglycan
- Polysiloxanes
- Polyisocyanides
- Polyisocyanates
- Polymethacrylates

Figure 23. Precursors - examples.

- N-carboxyanhydrides (NCA)
- N-thiocarboxyanhydrides (NTA)
- Amines
- Carboxylic acids
- Ketones
- Aldehydes
- Hydroxyls
- Thiols
- Esters
- Thioesters
- conjugated system of double bonds
- Alkyl halides
- Hydrazines
- N-hydroxysuccinimide esters
- Epoxides
- Haloacetyls
- UDP-activated saccharides
- Sulfides
- Cyanates
- Carbonylimidazole
- Thiazinanones
- Phosphines
- Hydroxylamines
- Sulfonates
- Activated nucleotides
- Vinylchloride
- Alkenes, quinones

Figure 24. Functional groups – examples.

- Hydroxyls
- Primary, secondary, tertiary amines
- Carboxylic acids
- Phosphates, phosphonates
- Sulfonates, sulfonamides
- Amides
- Carbamates
- Carbonates
- Ureas
- Alkanes, Alkenes, Alkynes
- Anhydrides
- Ketones
- Aldehydes
- Nitatrates, nitrites
- Imines
- Phenyl and other aromatic groups
- Pyridines, pyrimidines, purines, indole, imidazole, and heterocyclic bases
- Heterocycles
- polycycles
- Flavins
- Halides
- Metals
- Chelates
- Mechanism based inhibitors
- Small molecule catalysts
- Dextrins, saccharides
- Fluorescein, Rhodamine and other fluorophores
- Polyketides, peptides, various polymers
- Enzymes and ribozymes and other biological catalysts
- Functional groups for post-polymerization/post activation coupling of functional groups
- Drugs, e.g., taxol moiety, acyclovir moiety, "natural products"
- Supramolecular structures, e.g. nanoclusters
- Lipids
- Oligonucleotides, oligonucleotide analogs (e.g., PNA, LNA, morpholinos)

Figure 25. Polymers and the functional entities required to make them.

A.

Polymer	Functional Entity (reactive groups)	Linking molecule	Catalyst/reagent	General Figure	Specific Figure
polycyclic compound	di-coumarin		light	Fig. 11	Fig. 11, ex. 1
polyester	alcohol, carboxylic acid		carbodiimide	Fig. 12, Fig. 21	
polyester	hydroxyl, thioester			Fig. 14	
polyurea	di-amine	carbonyldiimidazole		Fig. 15	Fig 15, ex. 3
polyacetate	halogen, carboxylic acid		base	Fig. 12, Fig. 21	
polyacetate	alcohol, carboxylic acid		EDC or other carbodiimide	Fig. 12, Fig. 21	
polycarbamate	alcohol, isocyanate			Fig. 12, Fig. 21	
polycarbonate	diol	carbonyldiimidazole		Fig. 15	
peptoid	secondary amine, α-haloacetyl			Fig. 12, Fig. 21	
	primary amine, α-haloacetyl		alkylating agent	Fig. 12, Fig. 21	
glycogen	UDP-glucose		glycogen synthetase	Fig. 12, Fig. 21	
polysaccharide	UDP-activated saccharides		polysaccharide synthetases	Fig. 12, Fig. 21	
polysaccharide	glucosyl sulphide/sulfoxide activation system (Kahne glucosylation)		Kahne conditions	Fig. 12, Fig. 21	
polyamide	amine, N-hydroxysuccinimide ester			Fig. 12, Fig. 21	
polyamide	amine, carboxylic acid		carbodiimide	Fig. 12, Fig. 21	

Figure 25. Polymers and the functional entities required to make them.

B.

Polymer	Functional Entity (reactive groups)	Linking molecule	Catalyst/reagent	General Figure	Specific Figure
polyamide	di-amine	di-carboxylic acid	carbodiimide	Fig. 15	Fig. 15, ex. 2
polyamide	di-carboxylic acid	di-amine	carbodiimide	Fig. 15	
polyamide	amine, carboxylic acid	amine, carboxylic acid	carbodiimide	Fig. 16	
α-polypeptide	carboxyanhydride (5-membered ring)			Fig. 18	
β-polypeptide	carboxyanhydride (6 membered ring)			Fig. 18	Fig. 18, ex.1
γ-polypeptide	carboxyanhydride (7-membered ring)			Fig. 18	
α-polypeptide	2,2-diphenylthiazinanone (5-membered ring)			Fig. 18	
β-polypeptide	2,2-diphenylthiazinanone (6-membered ring)			Fig. 18	Fig. 18, ex.2
γ-polypeptide	2,2-diphenylthiazinanone (7-membered ring)			Fig. 18	
α-polypeptide	amine, thioester			Fig. 14	
β-polypeptide	amine, thioester			Fig. 14	Fig. 14, ex.1
γ-polypeptide	amine, thioester			Fig. 14	
ω-polypeptide	amine, thioester			Fig. 14	
polysulfonamide	amine, sulfonic acid		carbodiimide	Fig. 12, Fig. 21	
polyphosphonate	di-alcohol	activated phosphonate		Fig. 15	
polyphosphonate	di-alcohol	activated alkylphosphine	oxidating reagent, e.g. tert-butylhydroperoxide	Fig. 15	
polyphosphate	di-alcohol	diaminoalkoxy-phosphine	oxidating reagent, e.g. tertbutyl-hydroperoxide	Fig. 15	
polyphosphodiester	diol	diaminophosphine	oxidant (ButOOH)	Fig. 15	Fig. 15, ex. 5
polyphosphodiester	diaminophosphine	diol	oxidant (ButOOH)	Fig. 15	Fig. 15, ex. 6

Figure 25. Polymers and the functional entities required to make them.

C.

Polymer	Functional Entity (reactive groups)	Linking molecule	Catalyst/reagent	General Figure	Specific Figure
polyurethane	diamine	diisocyanate		Fig. 15	
polyether	epoxide			Fig. 18	Fig. 18, ex. 3
polythioether	thioepoxide			Fig. 18	
polydisulfide	thiol, thiol		oxidant	Fig. 11	
polyoxime	aldehyde, hydroxylamine			Fig. 12, Fig. 21	
polyimine	aldehyde, amine			Fig. 12, Fig. 21	
polyimine	aldehyde, amine			Fig. 15	Fig. 15, ex. 1
polynucleotides	nucleoside-5'-phosphoro-2-methylimidazolides			Fig. 12, Fig. 21	
polyamine	amine, alkyl sulfonate			Fig. 14	Fig. 14, ex.2
alkane	alkene			Fig. 17	Fig. 17, ex. 1
alkane	alkene			Fig. 17	Fig. 17, ex.2
polycycloalkane	di-diene	di-alkene (benzoquinone)		Fig. 15	Fig. 15, ex. 7
polyvinyl	vinylchloride unit			Fig. 17	
polystyrene	styrene-unit		radical initiator, AIBN	Fig. 17	
polyethylene	ethylene unit			Fig. 17	Fig. 17, ex. 1

Figure 26. Chemical Display – principle.

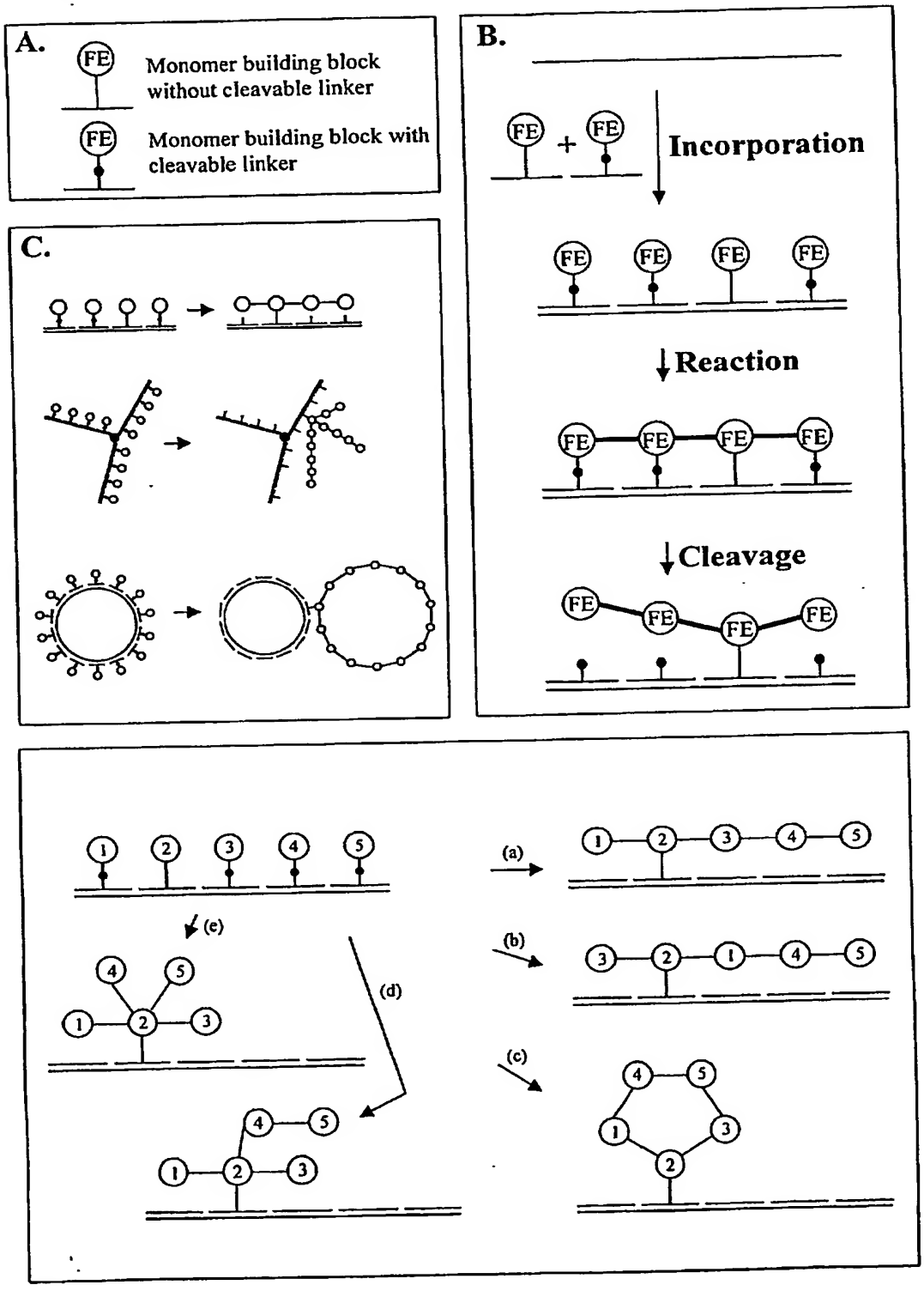


Figure 27. The number of reactive groups (X) can be higher than, equal to, or lower than the number of reactive groups (Y). When the number of (X) and (Y) are different, scrambling results.

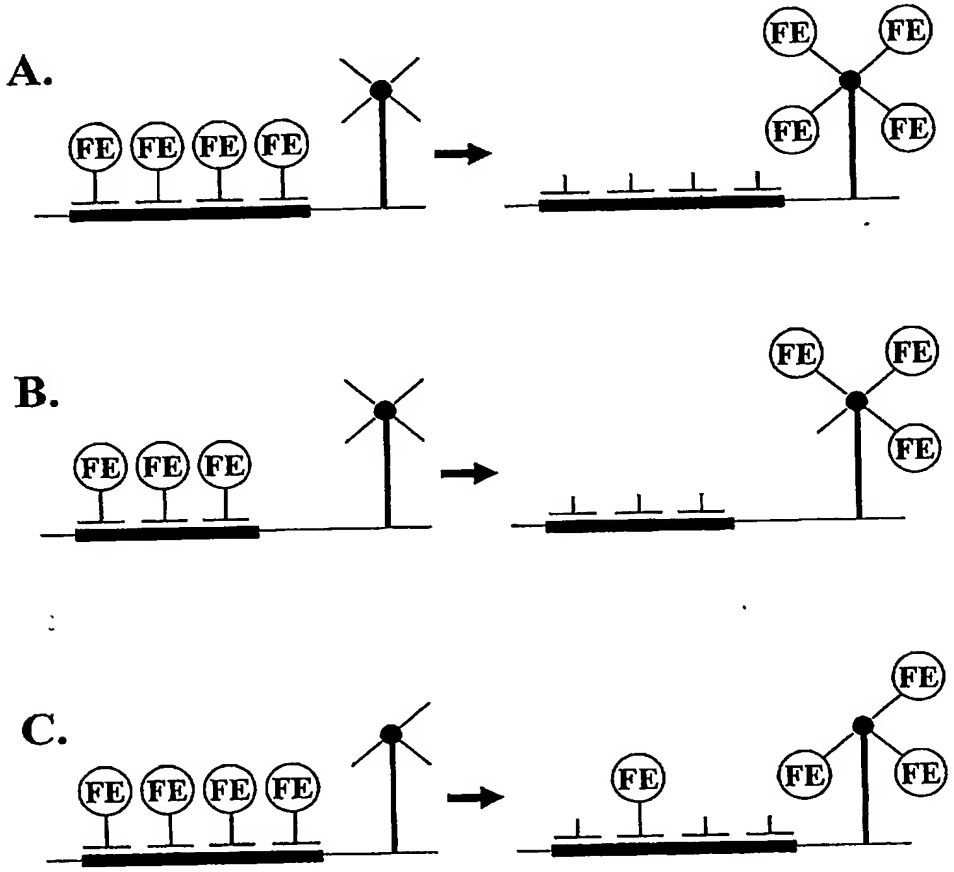


Figure 28. Monomer building blocks.

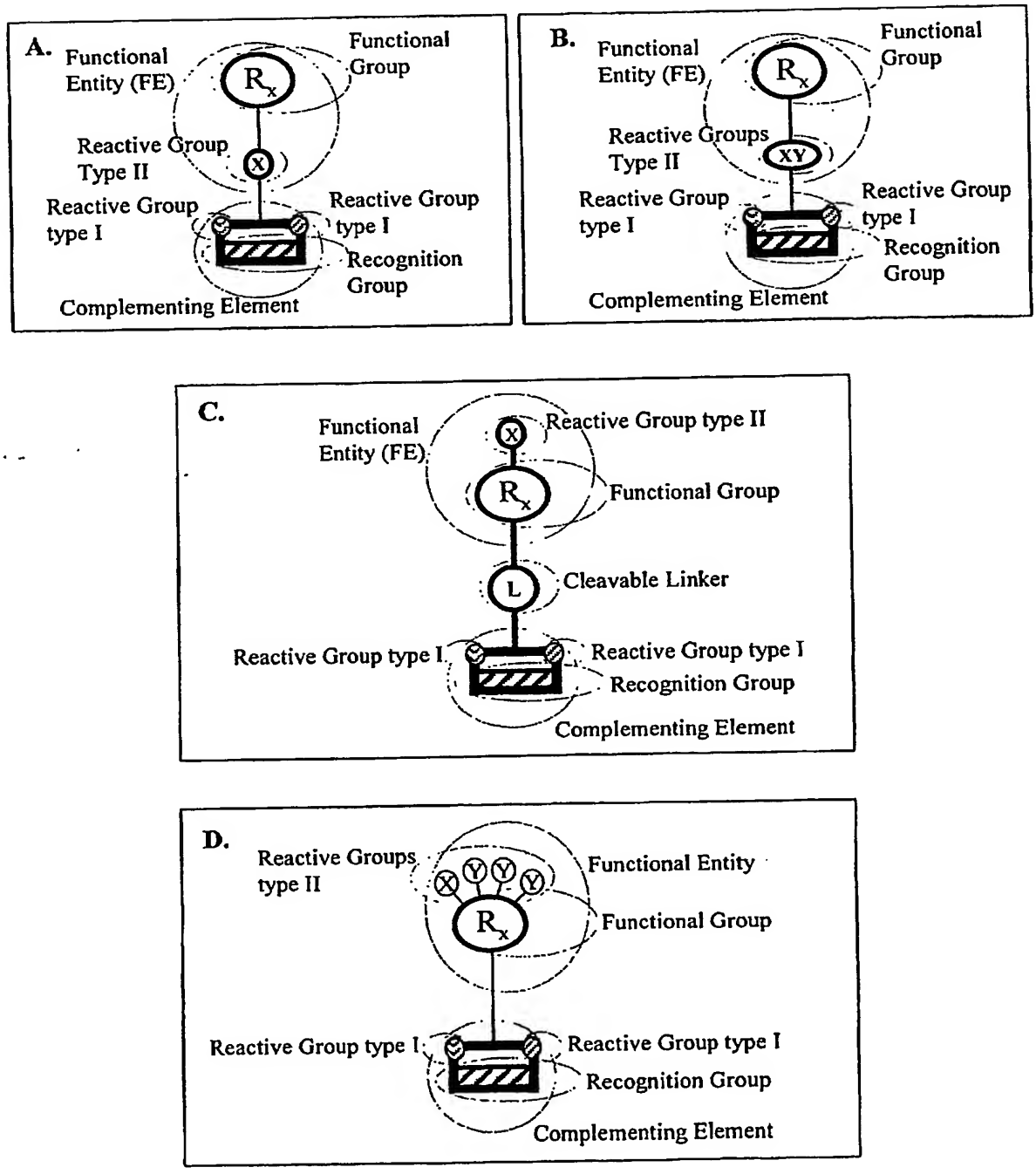


Figure 29. Templating involving simultaneous reaction and activation.

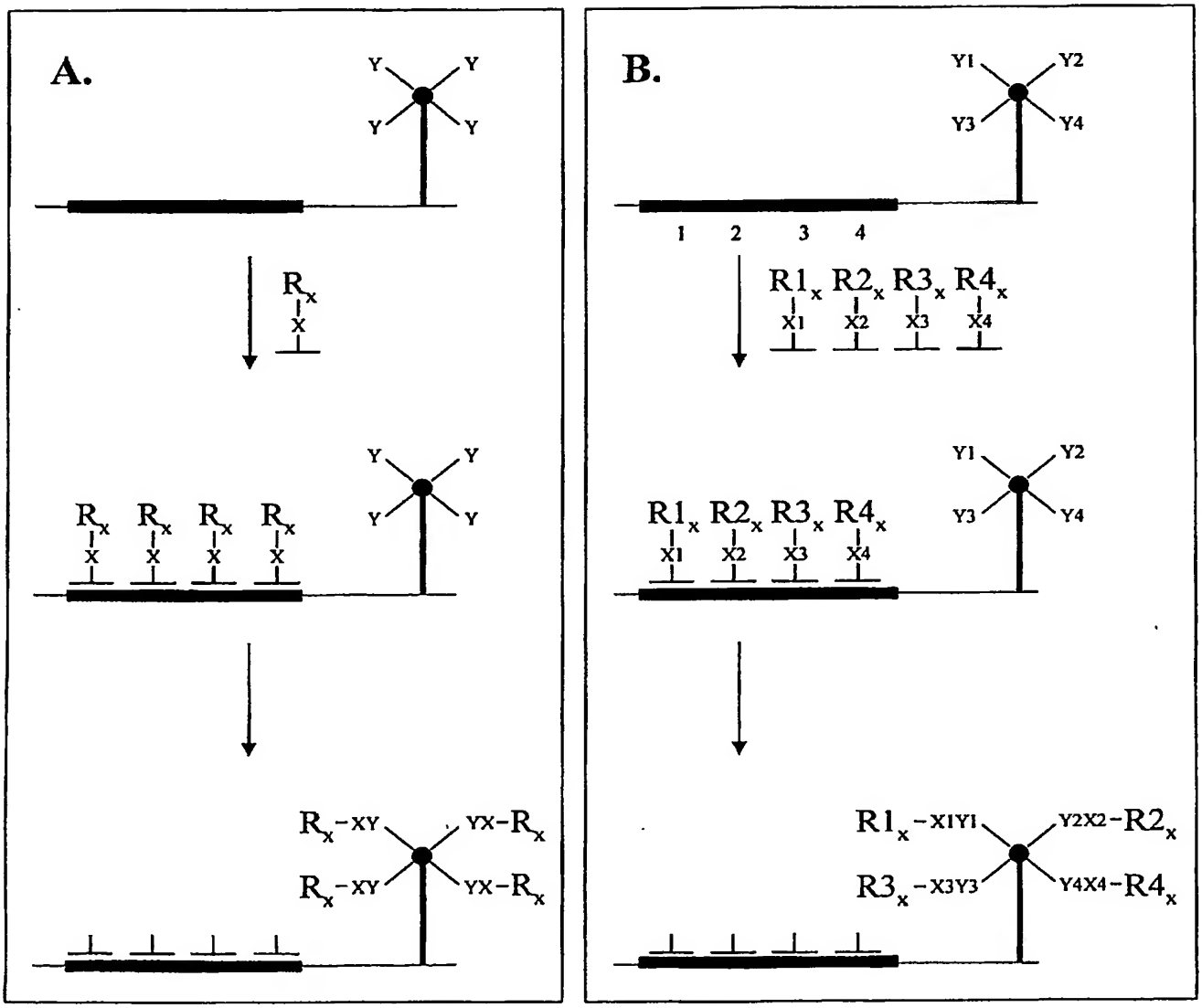
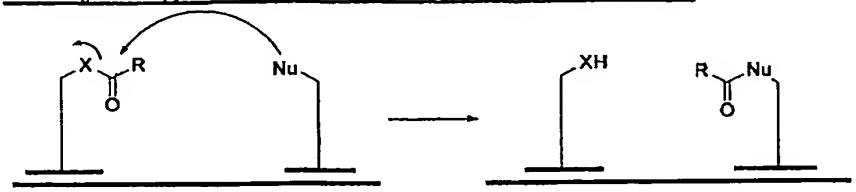


Fig 30. Reaction types allowing simultaneous reaction and activation.

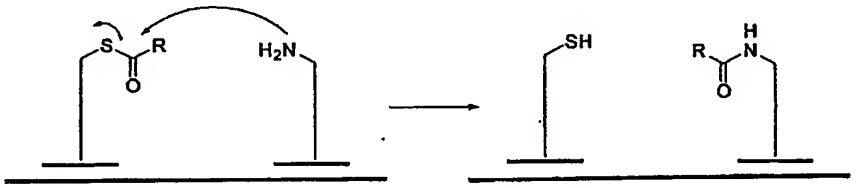
Nucleophilic substitution using activation of electrophiles

A. Acylating monomer building blocks - principle

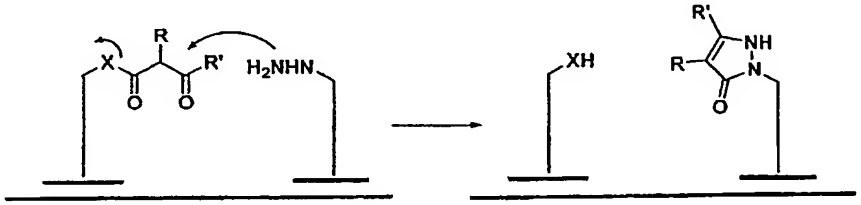


X = O, S Nu = Oxygen-, Nitrogen-, Sulfur- and Carbon Nucleophiles

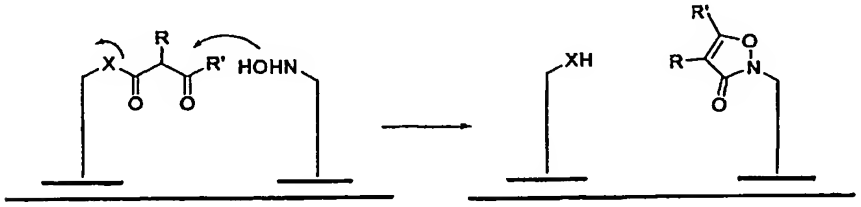
B. Acylation
Amide formation by reaction of amines with activated esters



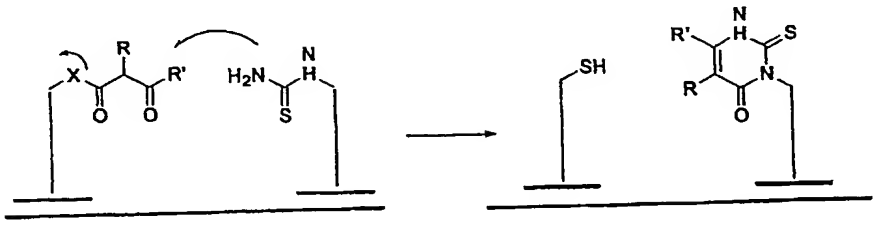
C. Acylation
Pyrazolone formation by reaction of hydrazines with β–Ketoesters



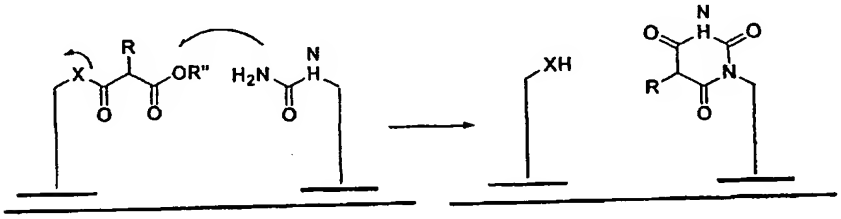
D. Acylation
Isoxazolone formation by reaction of hydroxylamines with β–Ketoesters



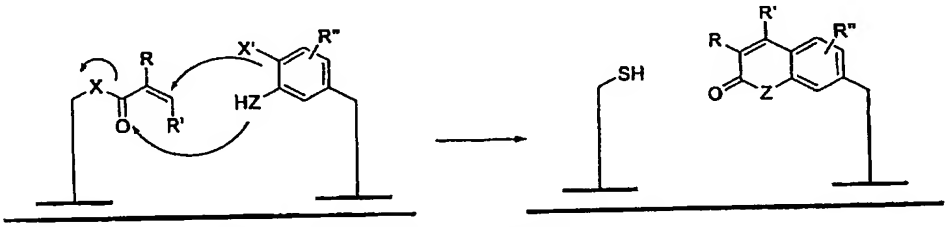
E. Acylation
Pyrimidine formation by reaction of thioureas with β–Ketoesters



F. Acylation
Pyrimidine formation by reaction of ureas with Malonates

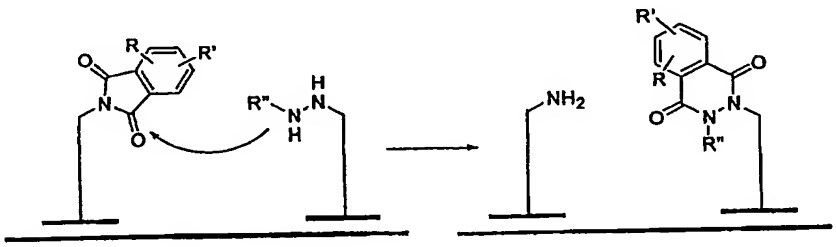


G. Acylation
Coumarine or quinolinon formation by a Heck reaction followed by a nucleophilic substitution

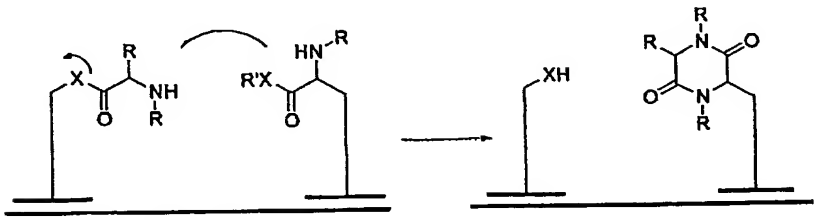


X = O,S X' = Halogen, OTf, OMs Z = O, NH

H. Acylation
Phthalhydrazide formation by reaction of Hydrazines and Phthalimides

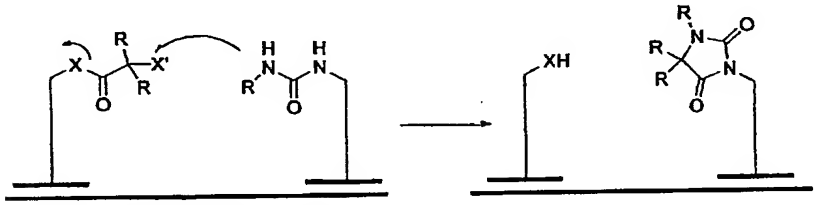


I. Acylation
Diketopiperazine formation by reaction of Amino Acid Esters



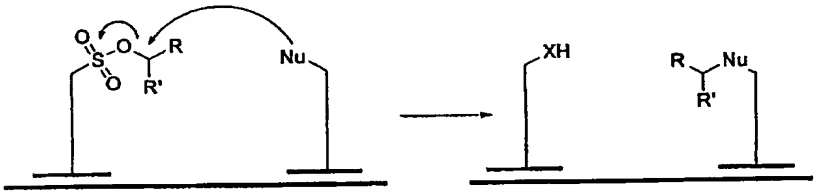
X = O, S R' = H, R

J. Acylation
Hydantoin formation by reaction of Urea and α-substituted Esters



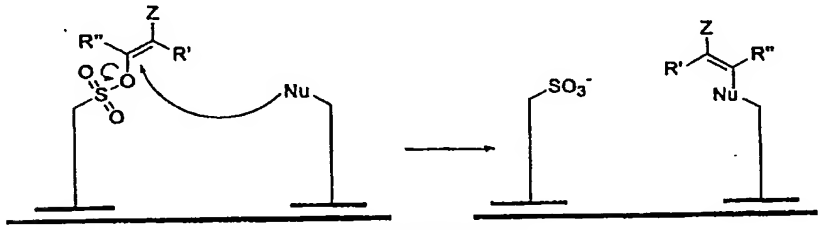
X = O, S X' = Hal, OTos, OMs, etc.

K. Alkylating monomer building blocks - principle
Alkylated compounds by reaction of Sulfonates with Nucleofiles



Nu = Oxygen- , Nitrogen- , Sulfur- and Carbon Nucleophiles

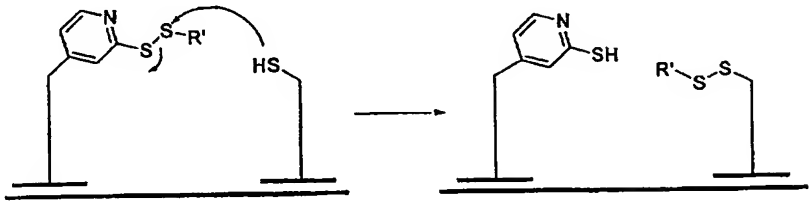
L. Vinylating monomer building blocks - principle



Z = CN, COOR, COR, NO_2, SO_2R, S(=O)R, SO_2NR_2, F
Nu = Oxygen- , Nitrogen- , Sulfur- and Carbon Nucleophiles

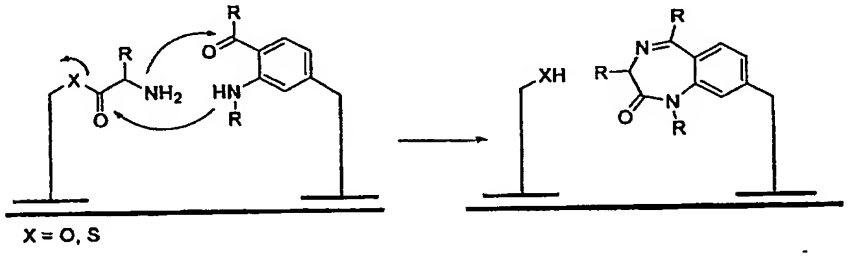
M. Heteroatom electrophiles

Disulfide formation by reaction of Pyridyl disulfide with Mercaptanes



N. Acylation

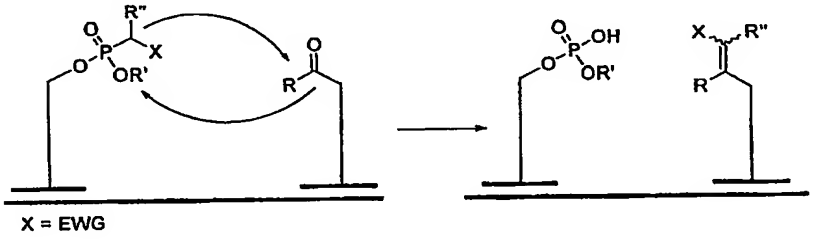
Benzodiazepinone formationby reaction of Amino Acid Esters and Amino Ketones



Addition to carbon-hetero multiple bonds

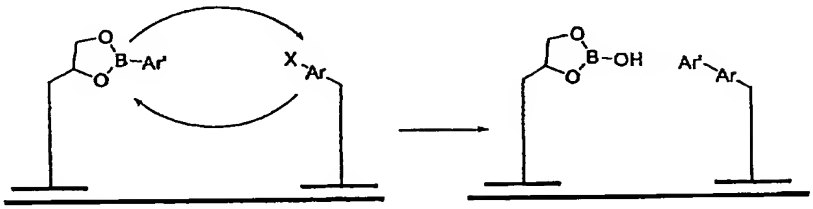
O. Wittig/Horner-Wittig-Emmons reagents

Substituted alkene formation by reaction of Phosphonates with Aldehydes or Ketones



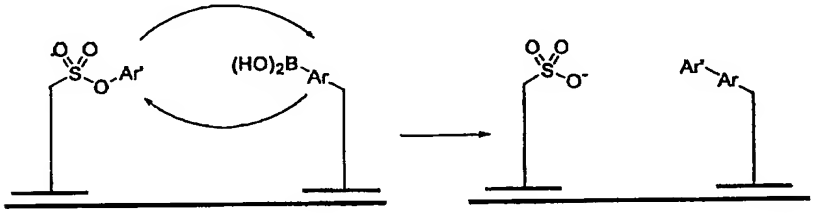
Transition metal catalysed reactions

P. Arylation
Biaryl formation by the reaction of Boronates with Aryls or Heteroaryls

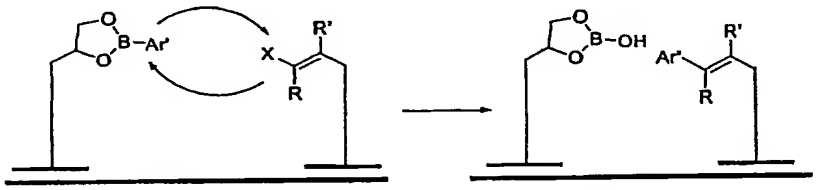


X = Halogen, OMs, OTf, OTos, etc

Q. Arylation
Biaryl formation by the reaction of Boronates with Aryls or Heteroaryls

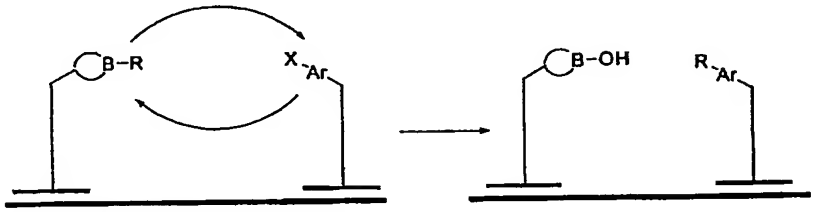


R. Arylation
Vinylarene formation by the reaction of alkenes with Aryls or Heteroaryls



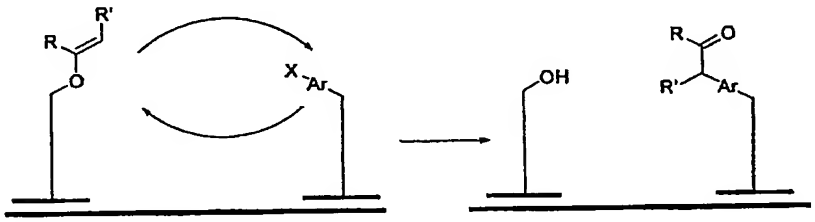
X = Halogen, OMs, OTf, OTos, etc

S. Alkylation
Alkylation of arenes/hetarens by the reaction with Alkyl boronates



X = Halogen, OMs, OTf, OTos, etc

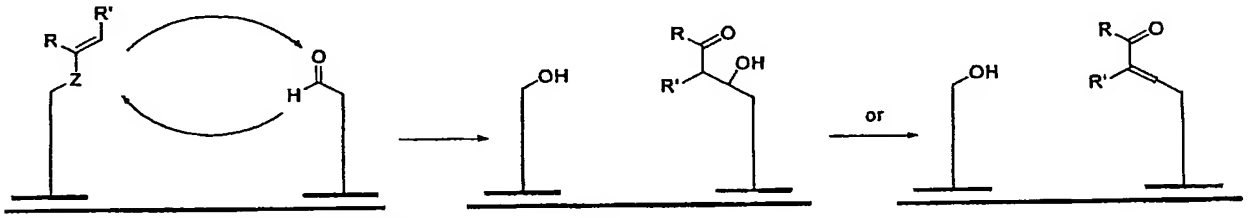
T. Alkylation
Alkylation of arenas/hetarenes by reaction with enolethers



X = Halogen, OMs, OTf, OTos, etc

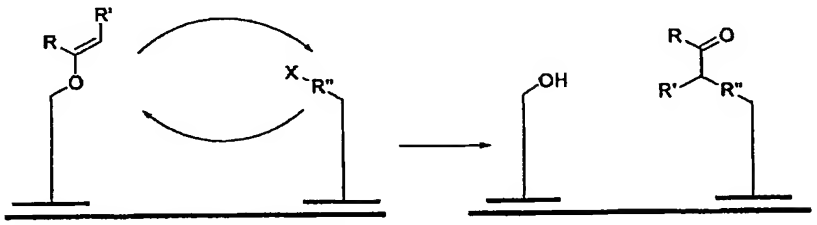
Nucleophilic substitution using activation of nucleophiles

U. Condensations
Alkylation of aldehydes with enolethers or enamines



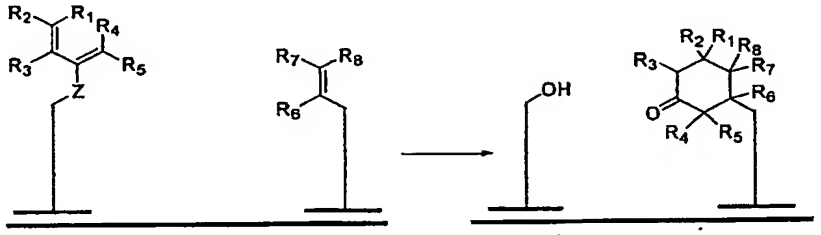
Z = NR, O; X = Halogen, OMs, OTf, OTos, etc

V. Alkylation
Alkylation of aliphatic halides or tosylates with enolethers or enamines

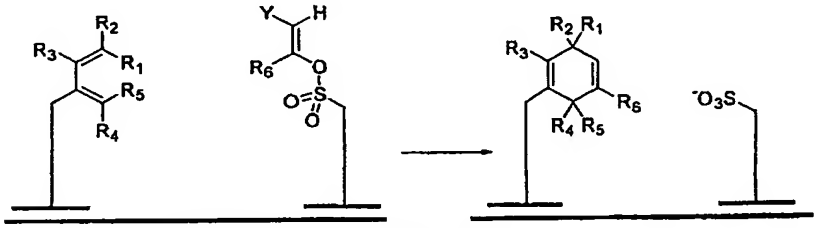


Cycloadditions

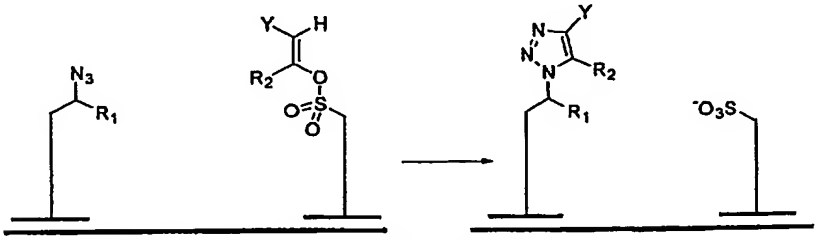
W. [2+4] Cycloadditions



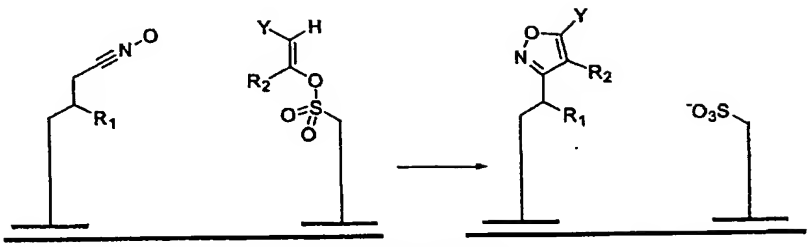
X. [2+4] Cycloadditions



Y. [3+2] Cycloadditions



Z. [3+2] Cycloadditions



Y, CN, COOR, COR, NO_2, SO_2R, S(=O)R, SO_2NR_2, F

Figure 31. Templating involving non-simultaneous reaction and activation.

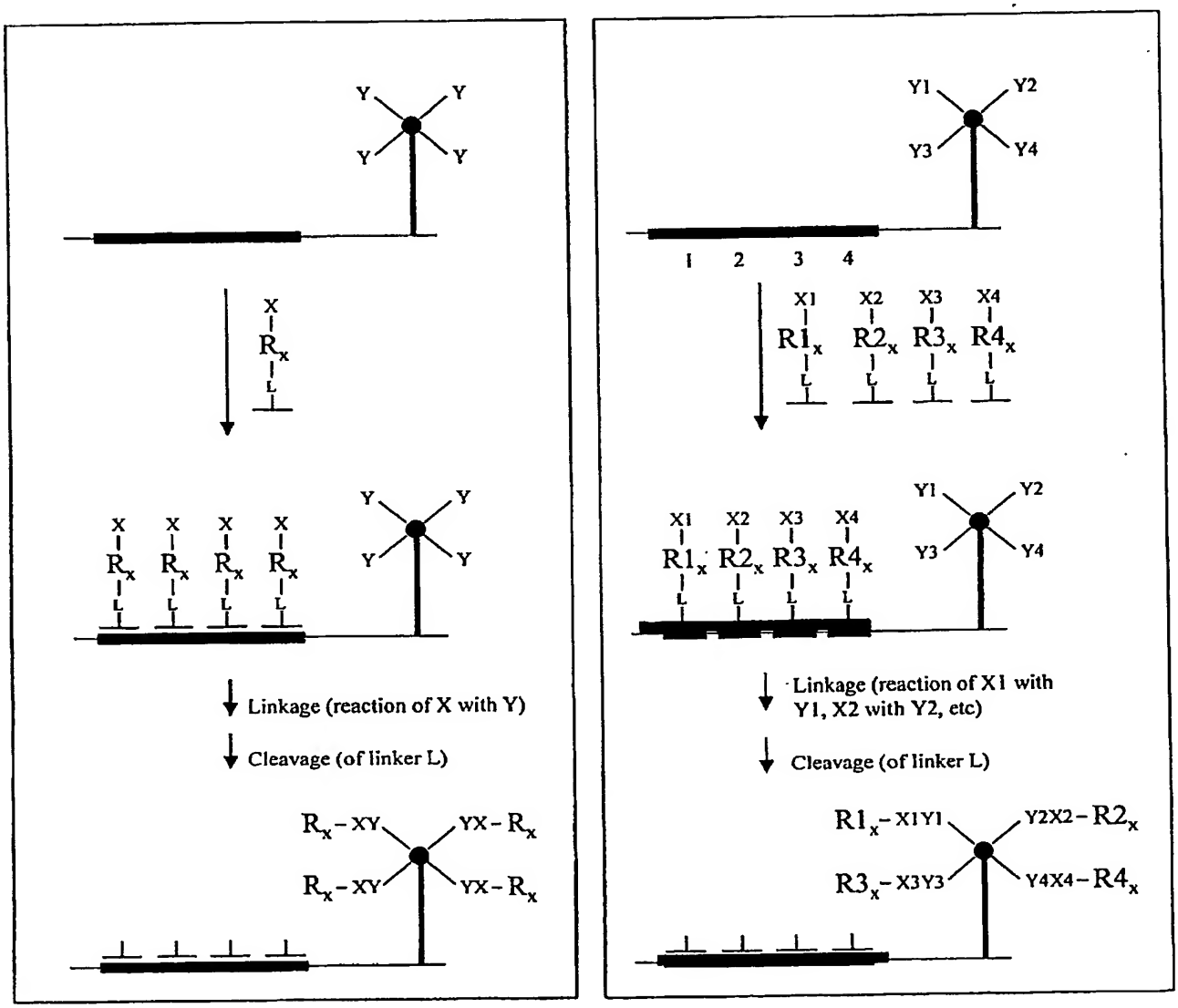
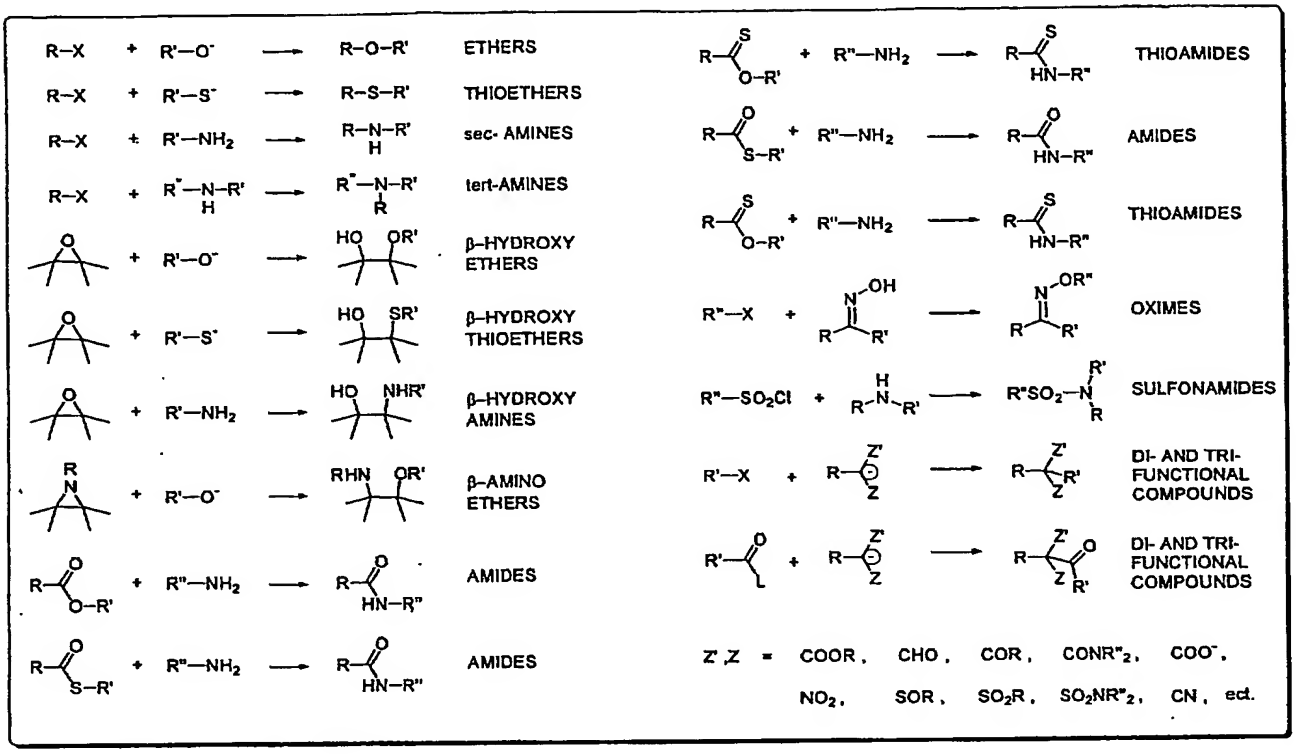
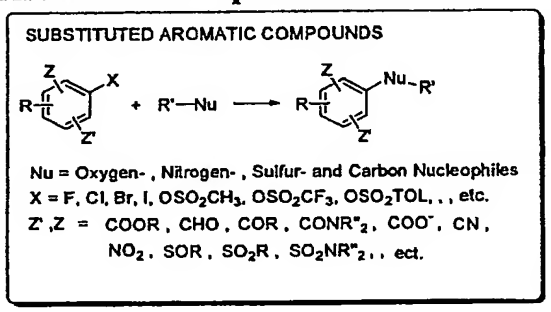


Figure 32. Pairs of reactive groups X,Y and the resulting bond XY.

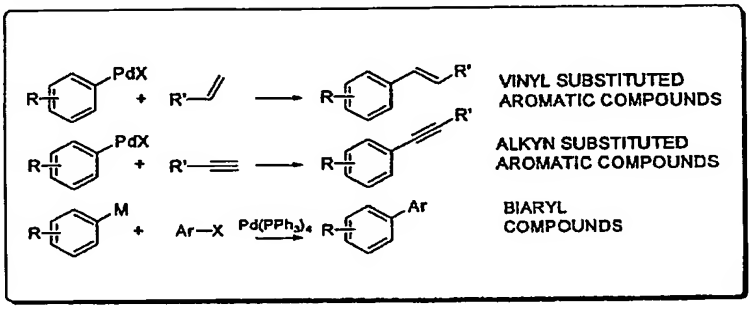
Nucleophilic substitution reaction



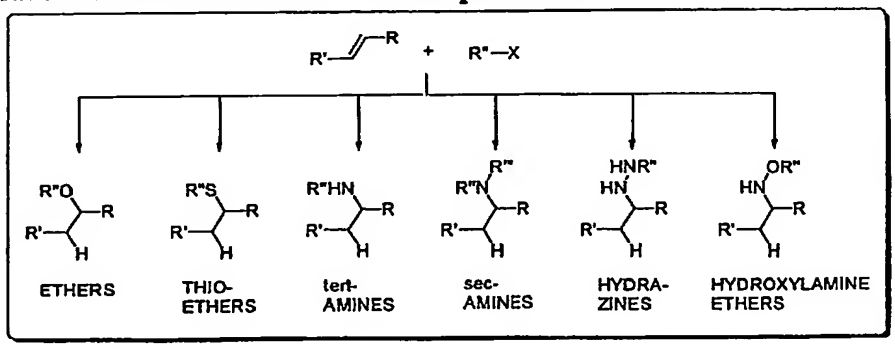
Aromatic nucleophilic substitution

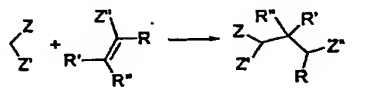


Transition metal catalysed reactions



Addition to carbon-carbon multiplebonds



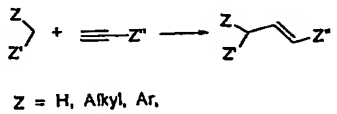


MULTI FUNCTIONAL COMPOUNDS

Z = H, Alkyl, Z', Ar

Z" = COOR , CHO , COR , $CONR"_2$, CN , NO_2 , SOR , SO_2R , $SO_2NR"_2$, , ect.

Z' = Z" R = R', = R", = Z



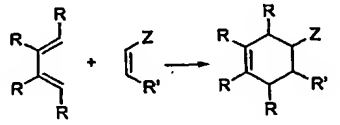
DI- AND TRI - FUNCTIONAL ALKENES

Z = H, Alkyl, Ar,

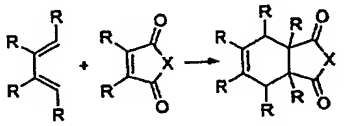
Z" = Z', Alkyl, Ar,

Z' = COOR, CHO, COR, $CONR"_2$, CN, NO_2, SOR, SO_2R, $SO_2NR"_2$, ect.

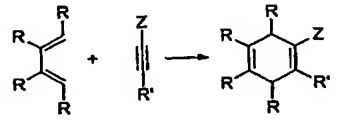
Cycloaddition to multiple bounds



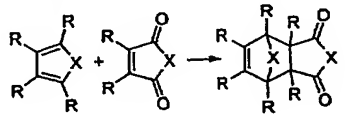
SUBSTITUTED CYCLOALKENES



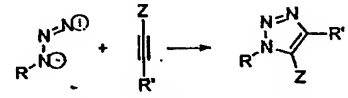
SUBSTITUTED CYCLOALKENES



SUBSTITUTED CYCLODIENES



SUBSTITUTED CYCLOALKENES

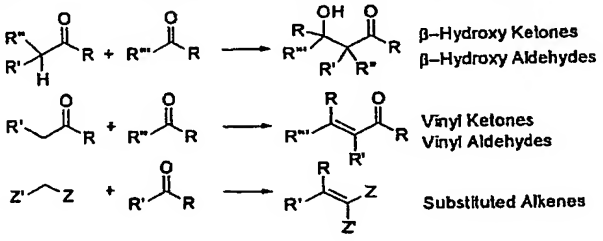


SUBSTITUTED 1,2,3-TRIAZOLES

Z = COOR , CHO , COR , COOH COAr CN , NO_2 , Ar, CH_2OH, CH_2NH_2, CH_2CN, SOR, SO_2R etc.

R = H, Alkyl, Ar, Z X = O, NR, CR_2, S,

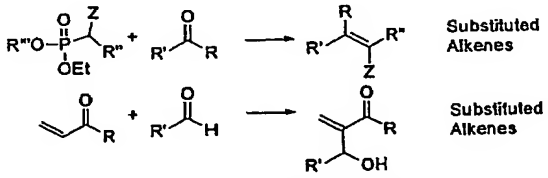
Addition to carbon-hetero multiple bonds



β–Hydroxy Ketones
β–Hydroxy Aldehydes

Vinyl Ketones
Vinyl Aldehydes

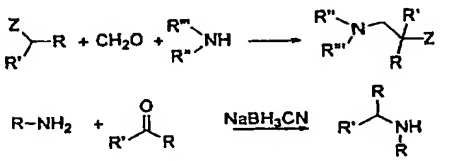
Substituted Alkenes



Substituted Alkenes

Substituted Alkenes

Z, Z' = COOR, CHO, COR, $CONR"_2$, CN, NO_2,SOR, SO_2R, $SO_2NR"_2$, ect. R^{n} = H, Alkyl, Aryl



Substituted Amines

Z = COOR, CHO, COR, SOR, SO_2R, CN, NO_2, ect.

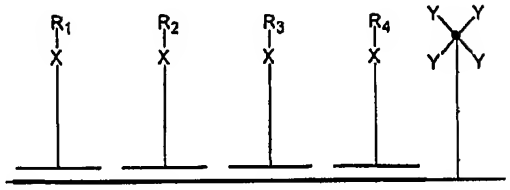
R = R', H, Alkyl, Ar,

R" = R"', H, Alkyl, COR,

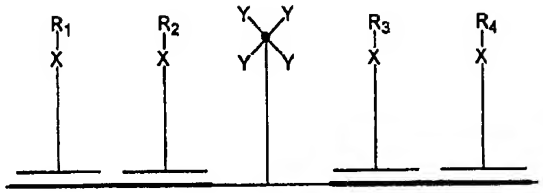
Substituted Amines

33. Anchorage - Examples

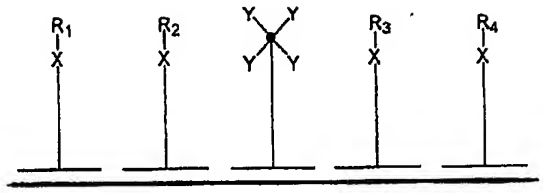
A.

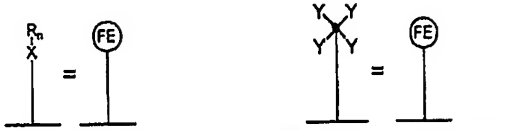


B.



C.

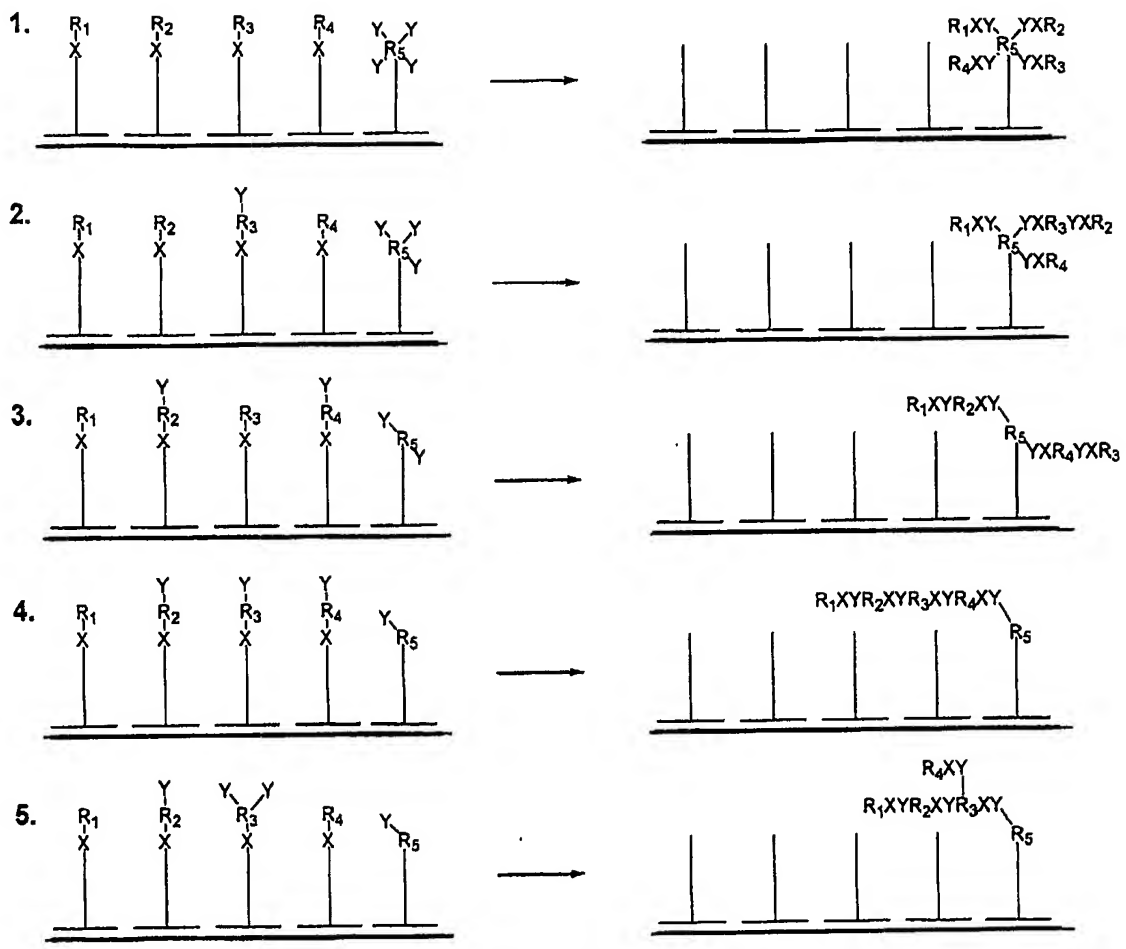




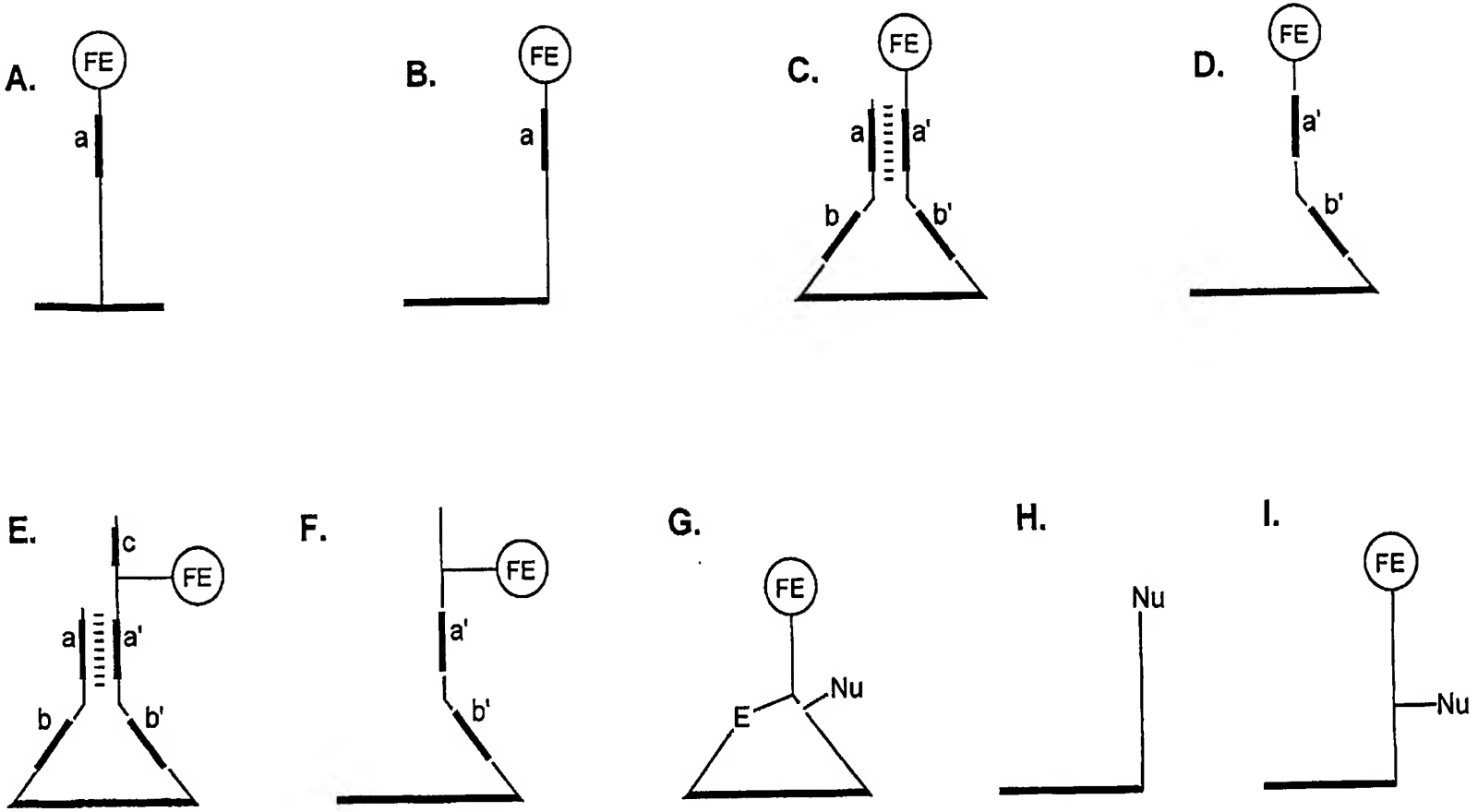
Monomer Building Block Type I

Monomer Building Block Type II

34. Branching and Scrambling



35. Monomer Building Blocks – Examples of Linker Design



36. Formation of Monomer Building Blocks – Examples

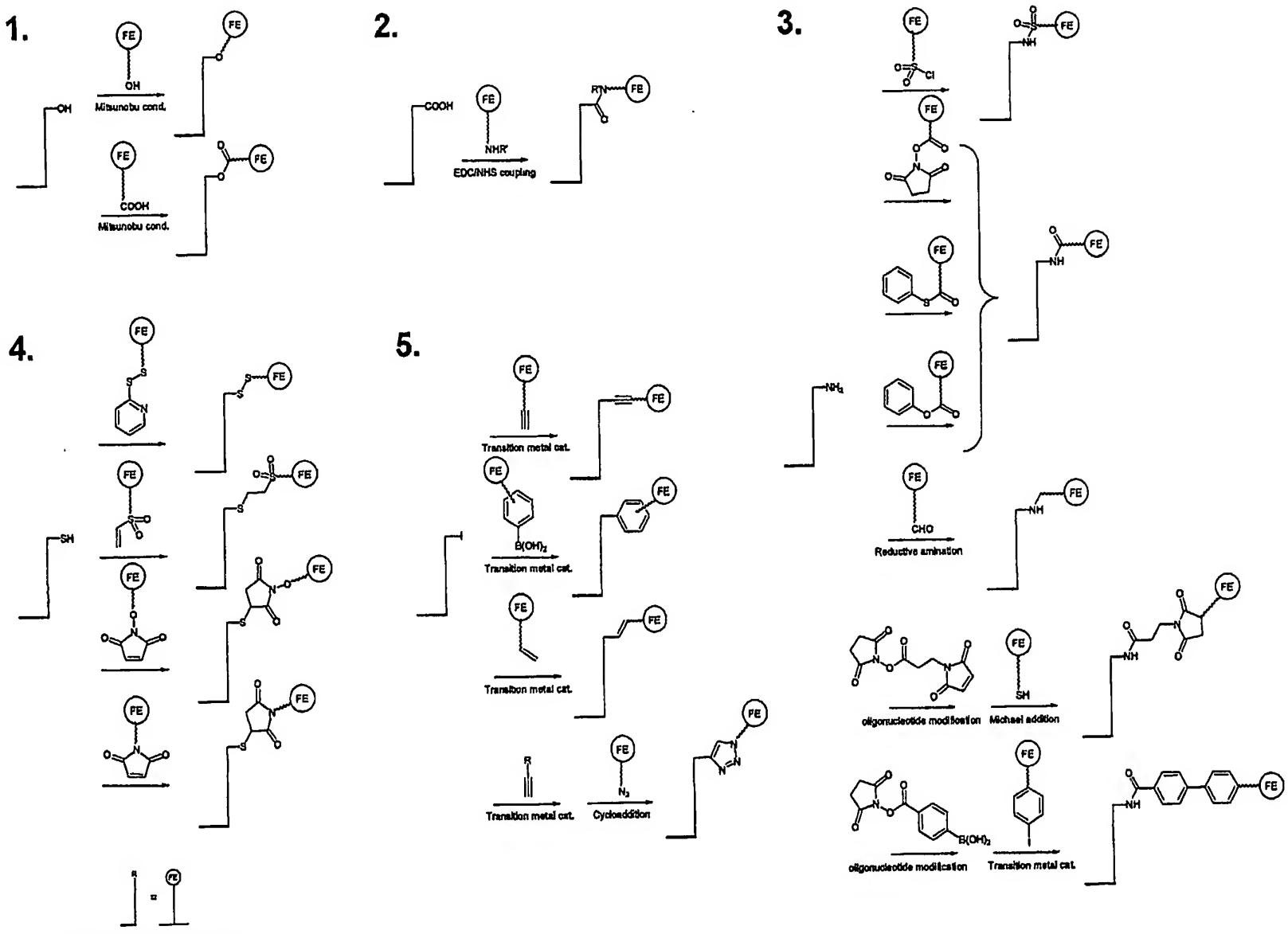
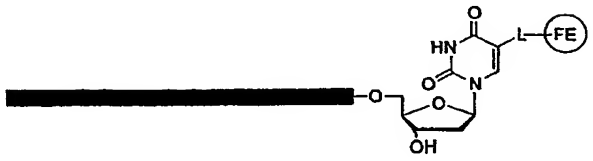
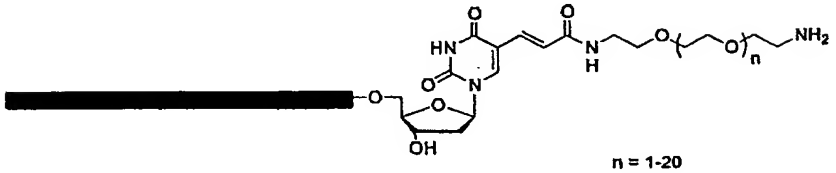


Figure 37. Oligonucleotide-based monomer building blocks. Examples of linker and functional entity (FE) design.

A

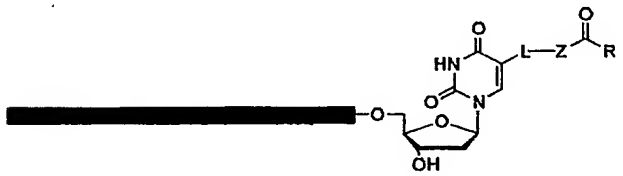


B



n = 1-20

C



Z = O, S

D

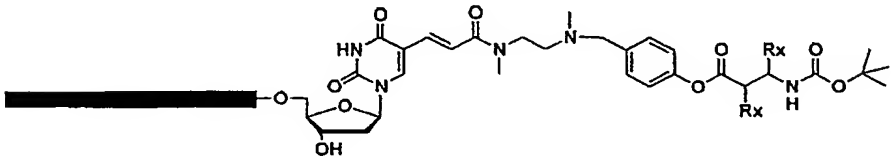
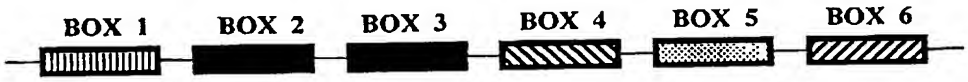


Figure 38. An oligonucleotide-based monomer building block. Example of complementing element design, allowing for high monomer diversity.

A.



BOX	sequence	monomer diversity
1	XXXXXATATTTXXXXX	1024
2	XXXATTTTAXXXXXXX	1024
3	XXXATTTTAXXXXXXX	1024
4	XXATXXATXXATXXXX	1024
5	GCCCGATTAAAXXCCG	4
6	XAXAXTTXTTXXXGGG	128

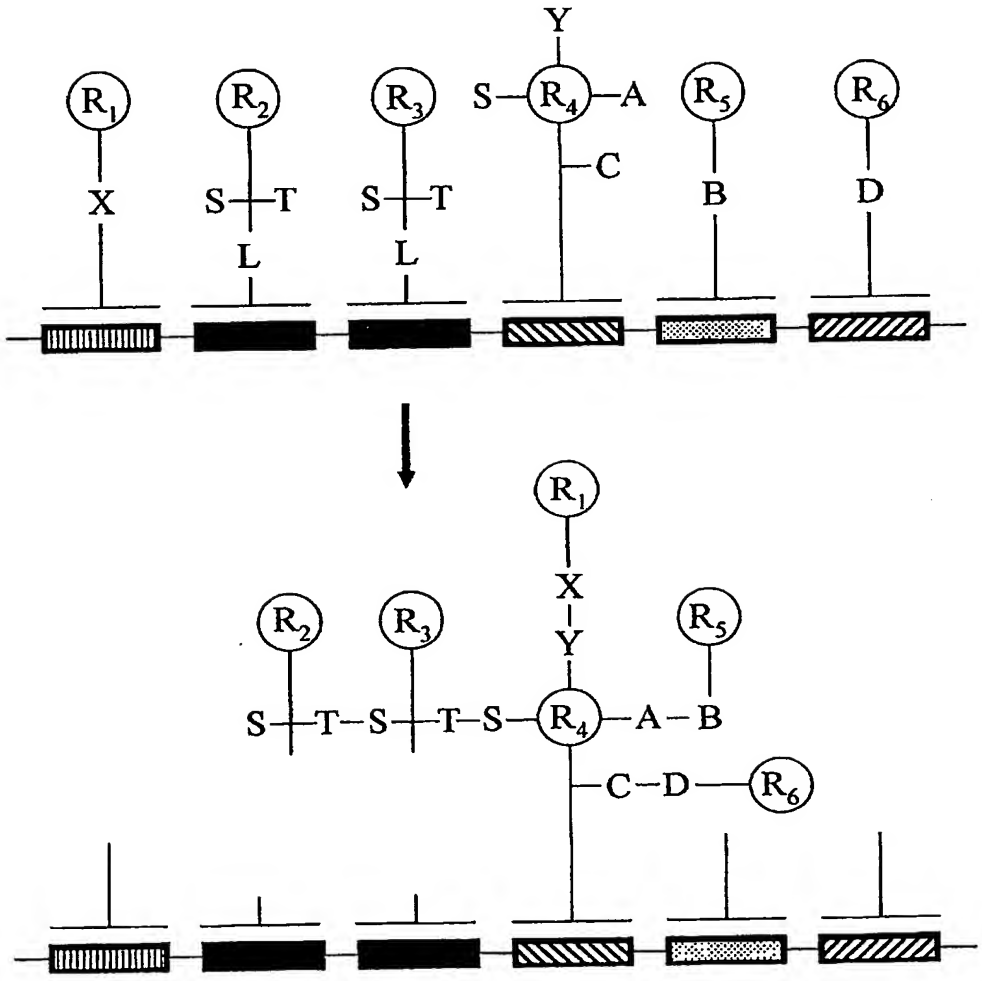
X = G or C

B.

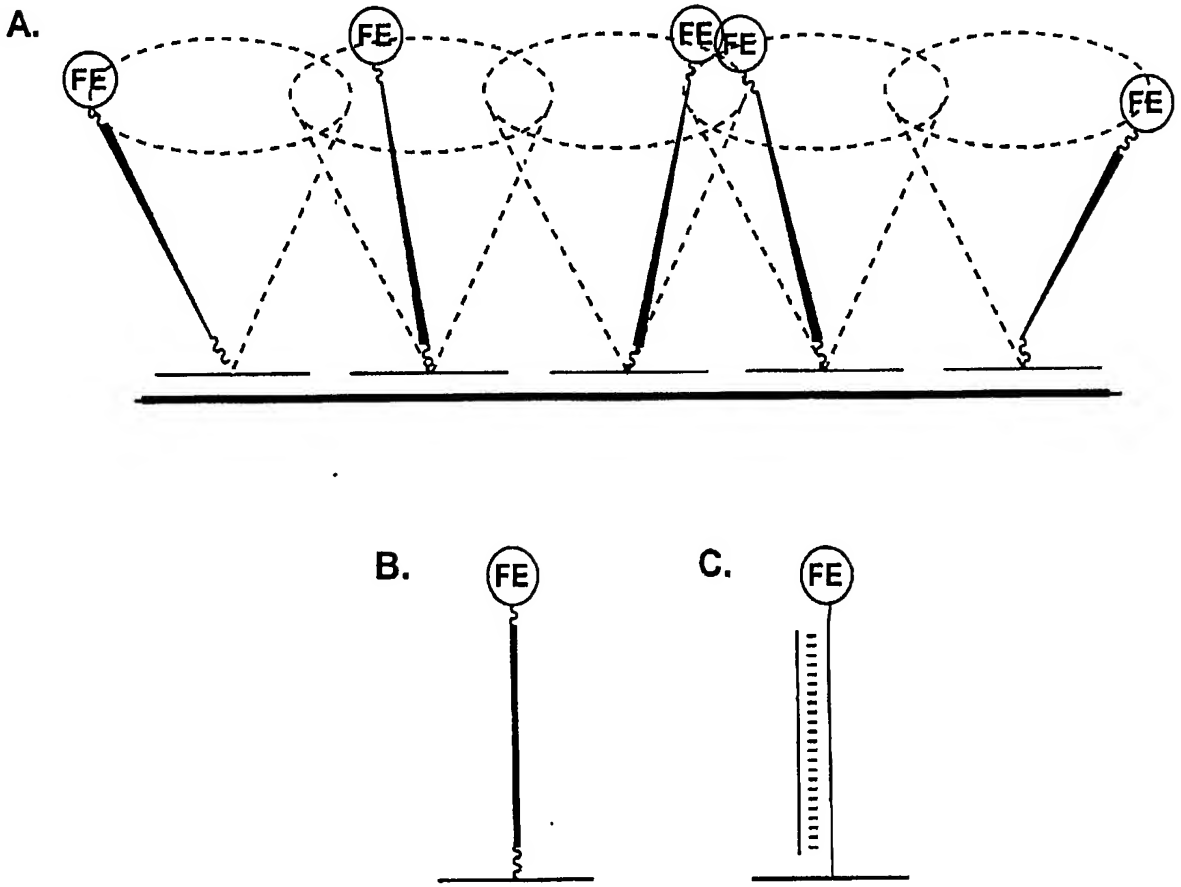
Coding Element (BOX 1)	GCGCGATATTTGGGCC
Complementing Element	CGCGCTATAAACCCGG
Coding Element (BOX 6)	GAGAGTTCTTCGCGGG
Complementing Element	CTCTCAAGAAGCGCCC

Figure 38, continued.

C.

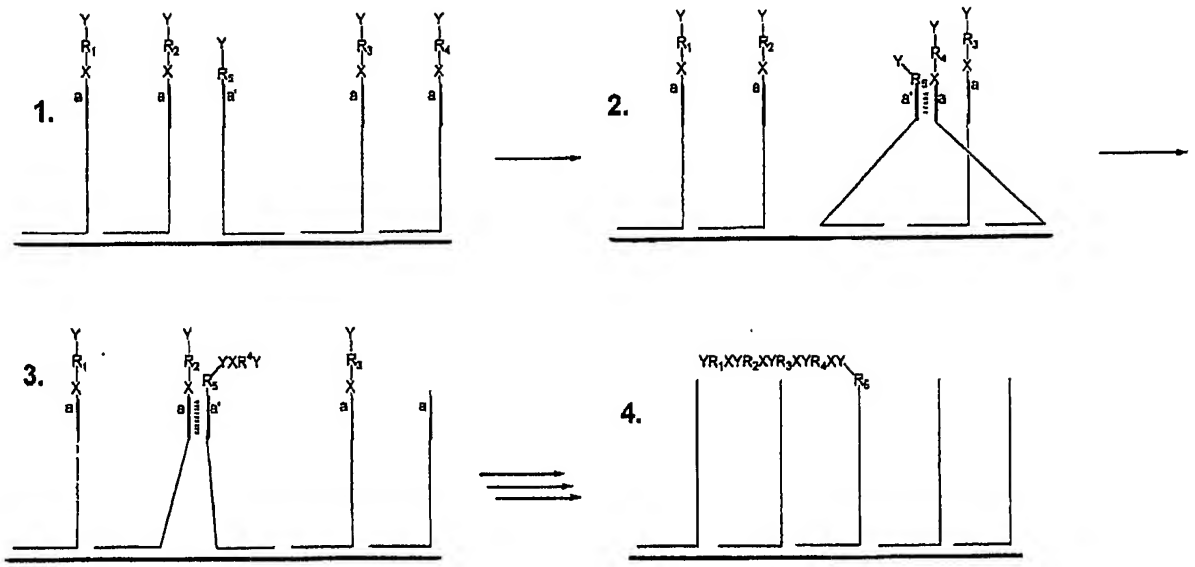


39. Use of Rigid or Partially Ridig Linkers



40. Use of Zipper Box

A.



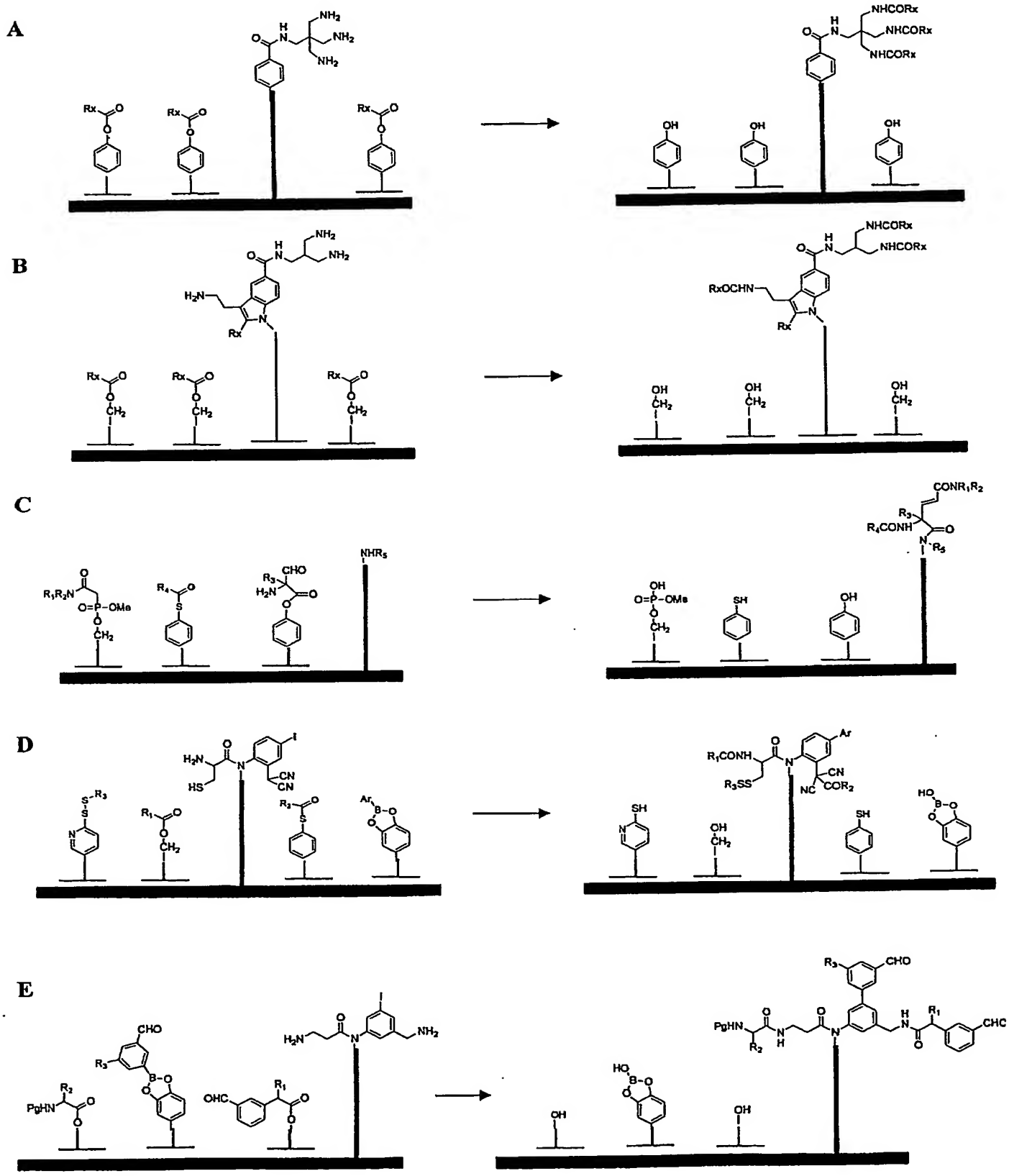
B. Example of a Zipping oligonucleotide setup

X= Carboxy-dT Glen cat.no. 10-1035-
Z= Amino-Modifier Glen C6 dT 10-1039-

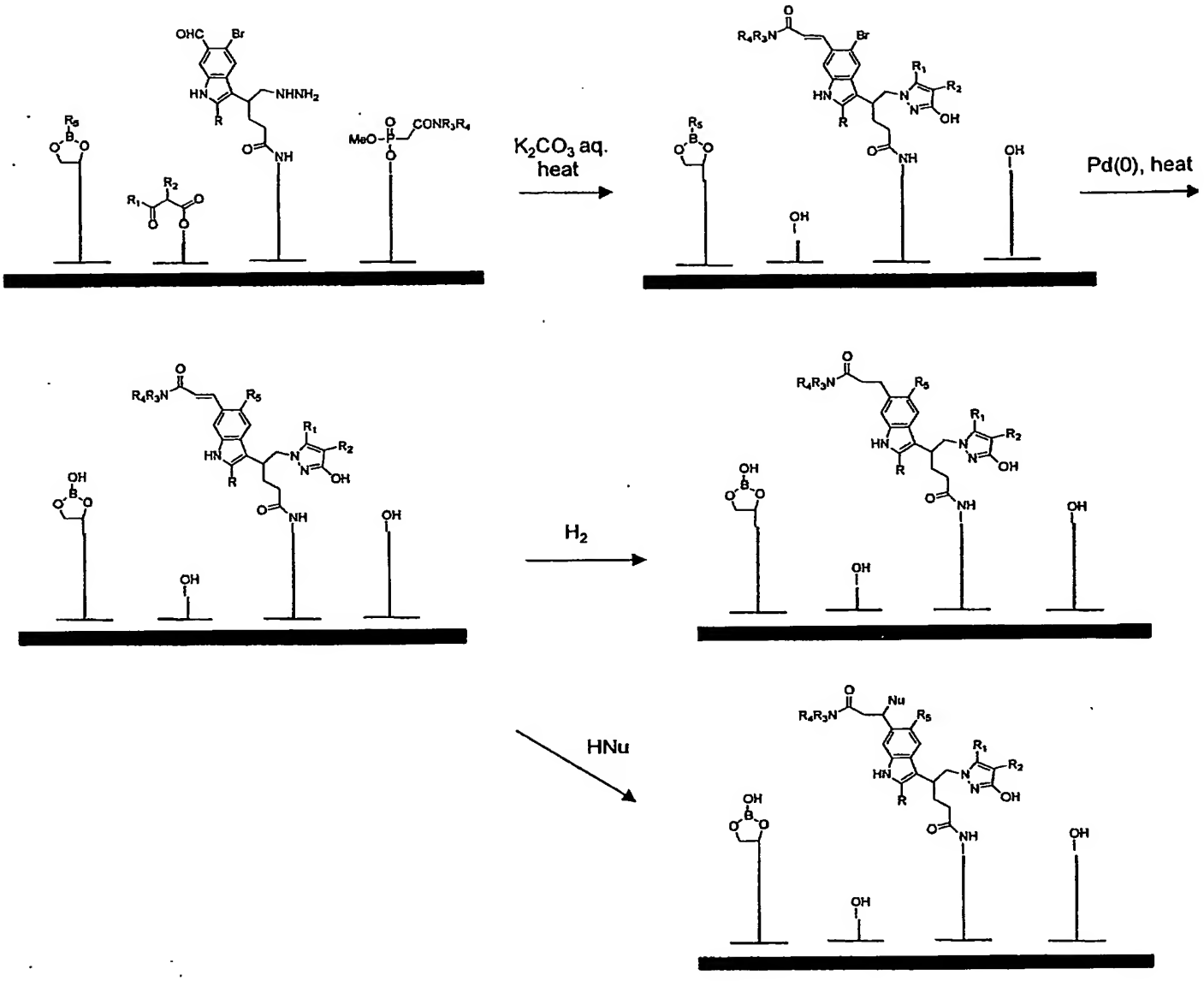
Oligo1
5'-CGACCTCTGGATTGCATCGGTCATGGCTGACTGTCCGTCGAATGTGTCCAGTTACX
Annealing region Linker region Zipper region

Oligo2
5'-ZGTAACACCTGTGTAAGCTGCCTGTCAGTCGGTACTGACCTGTCGAGCATCCAGCT
Zipper region Linker region Annealing region

Figure 41. Templated synthesis of organic compounds – examples.

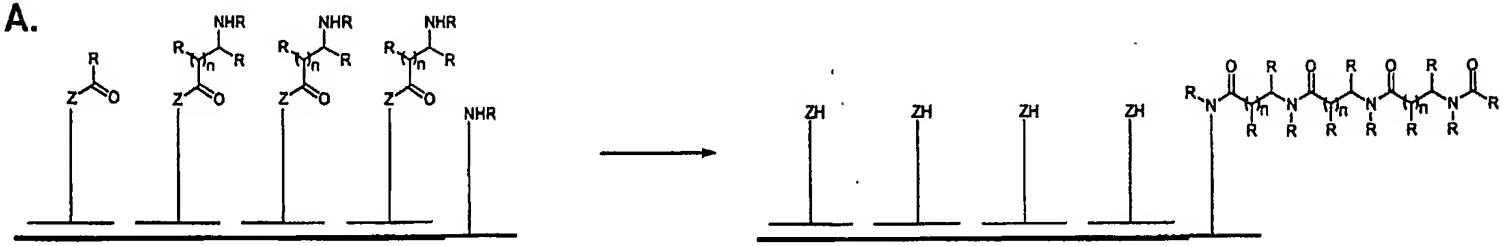


F



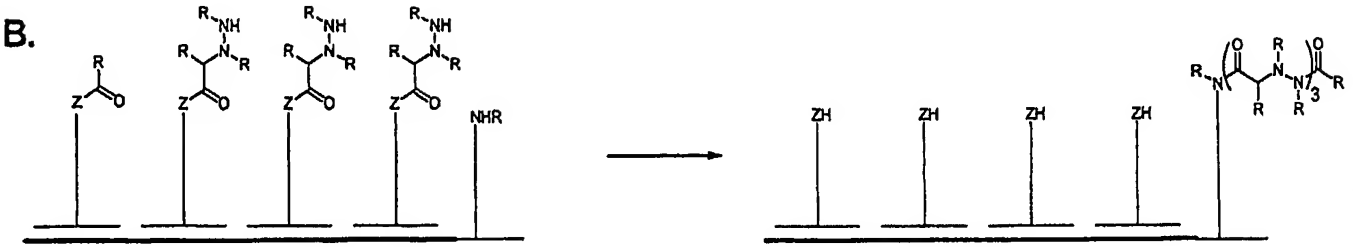
42. Polymer encoding – Examples: peptide (α, β), peptoid or hydrazino peptide

A.



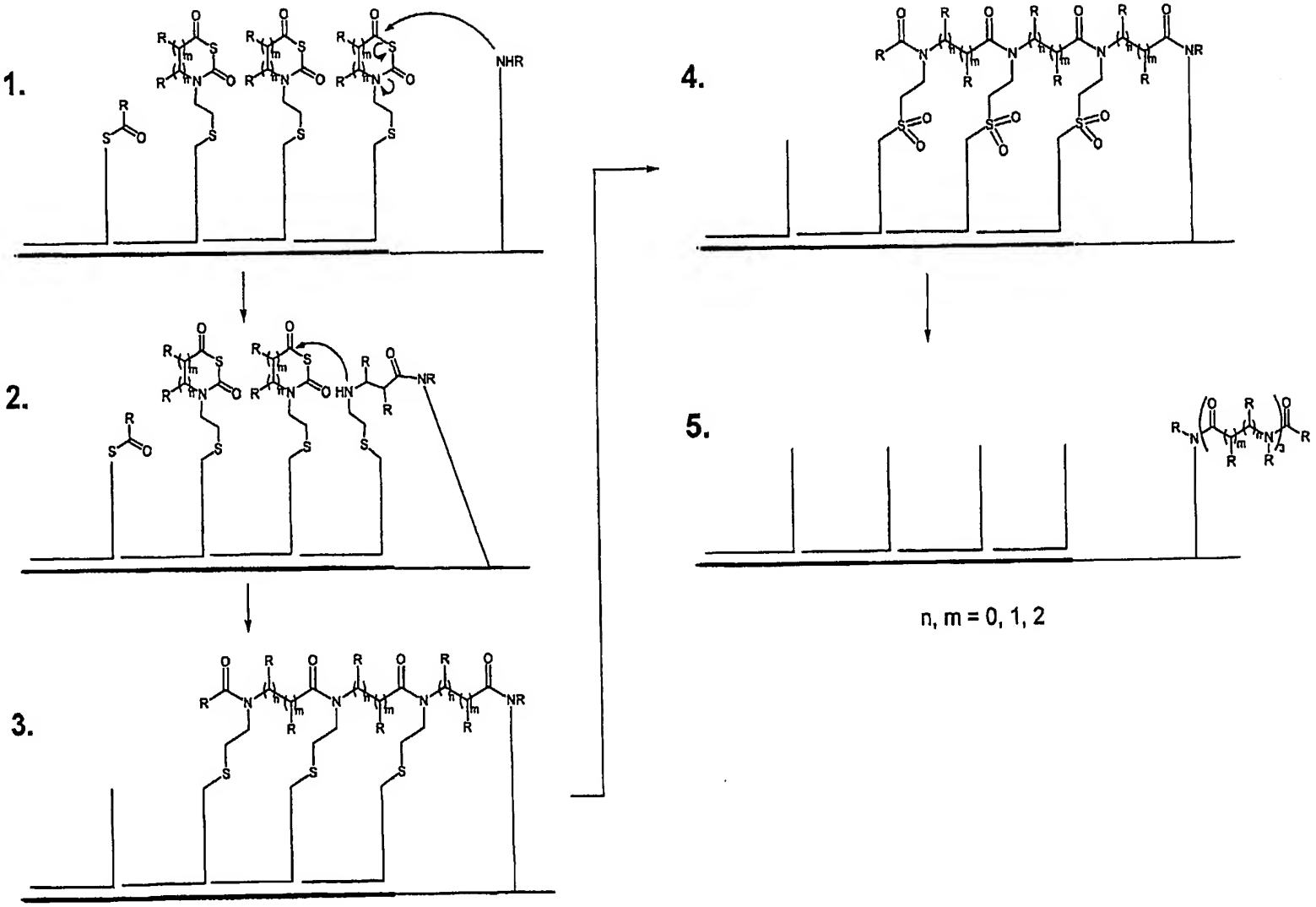
Z = O, S
n = 0, 1

B.

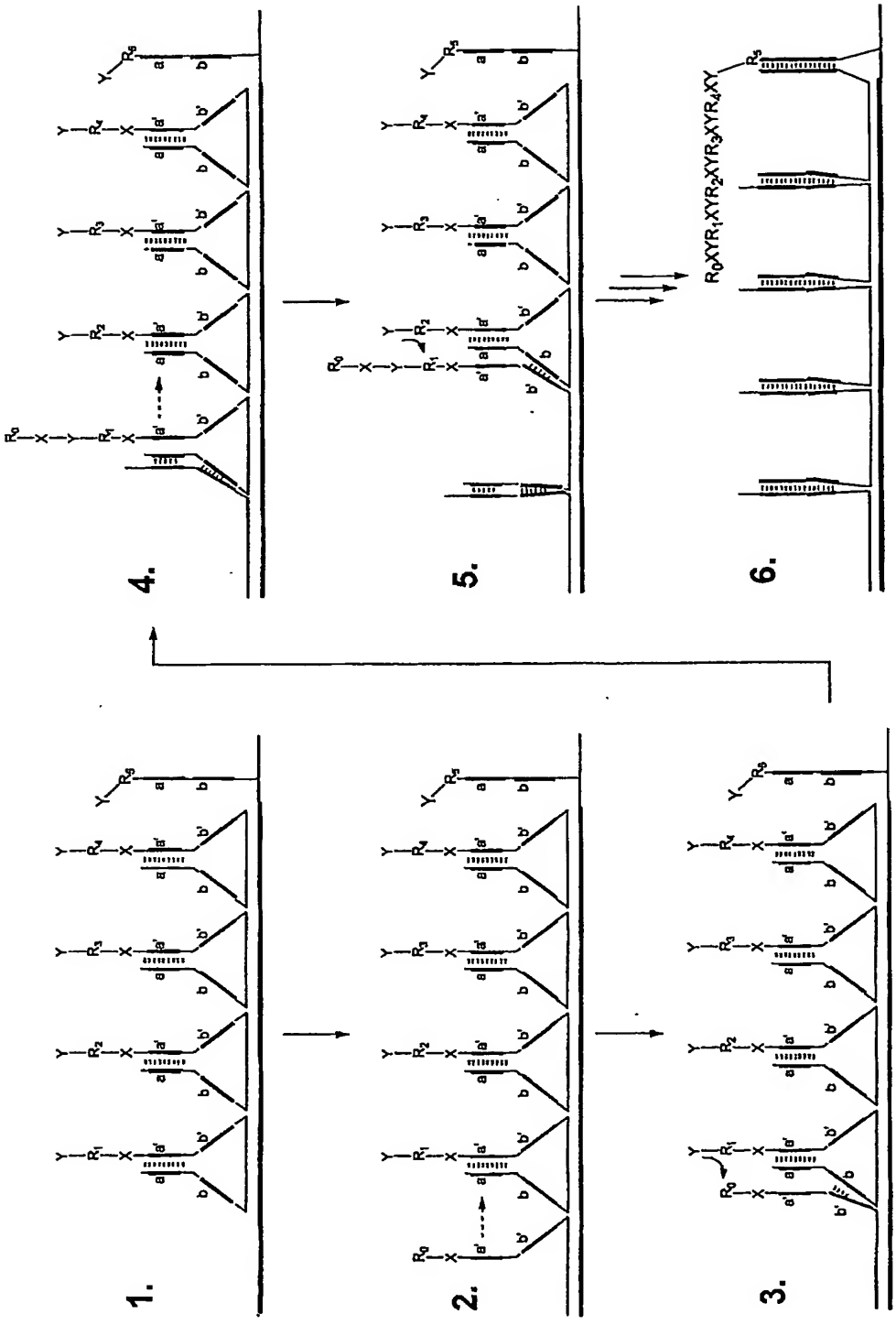


Z = O, S

43. Polymer encoding – Examples: α, β, γ, ω-peptide through use of NTA units

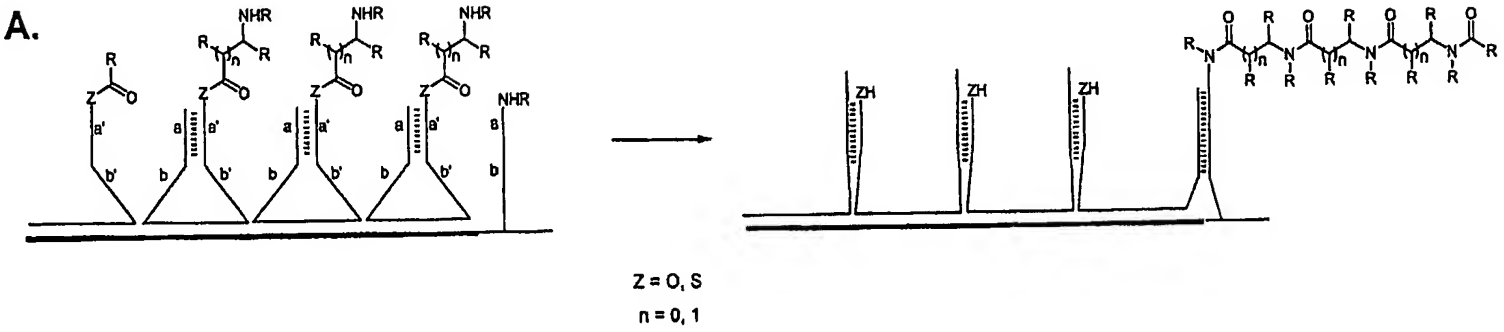


44. Directional encoding by hybridization and release



44. Examples 1: Directional polymer encoding of peptides (α, β), peptoids or hydrazinopeptides

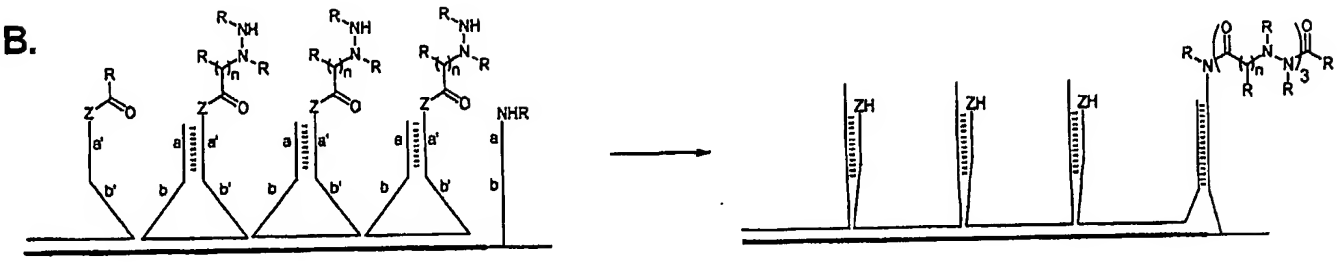
A.



Z = O, S

n = 0, 1

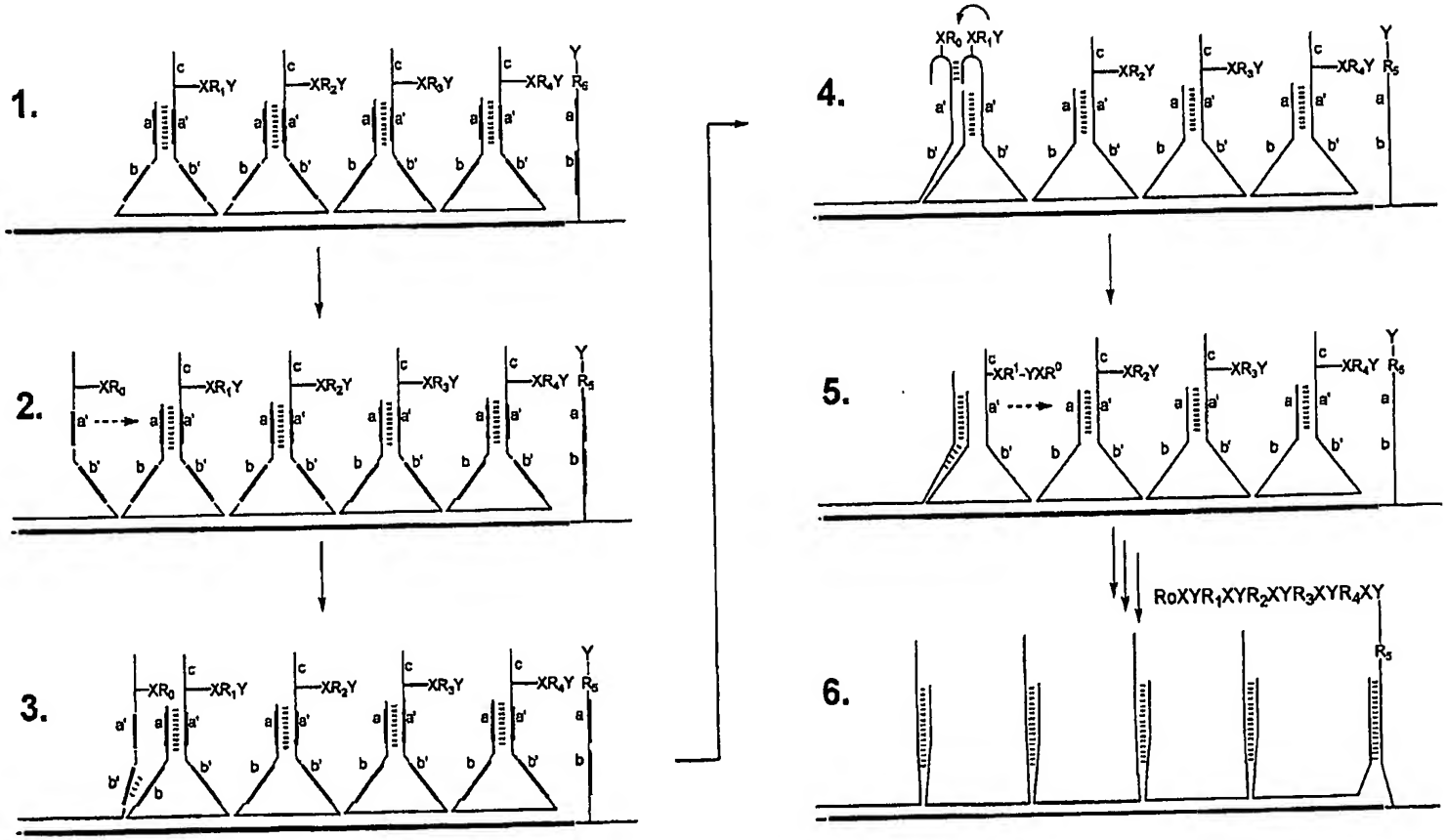
B.



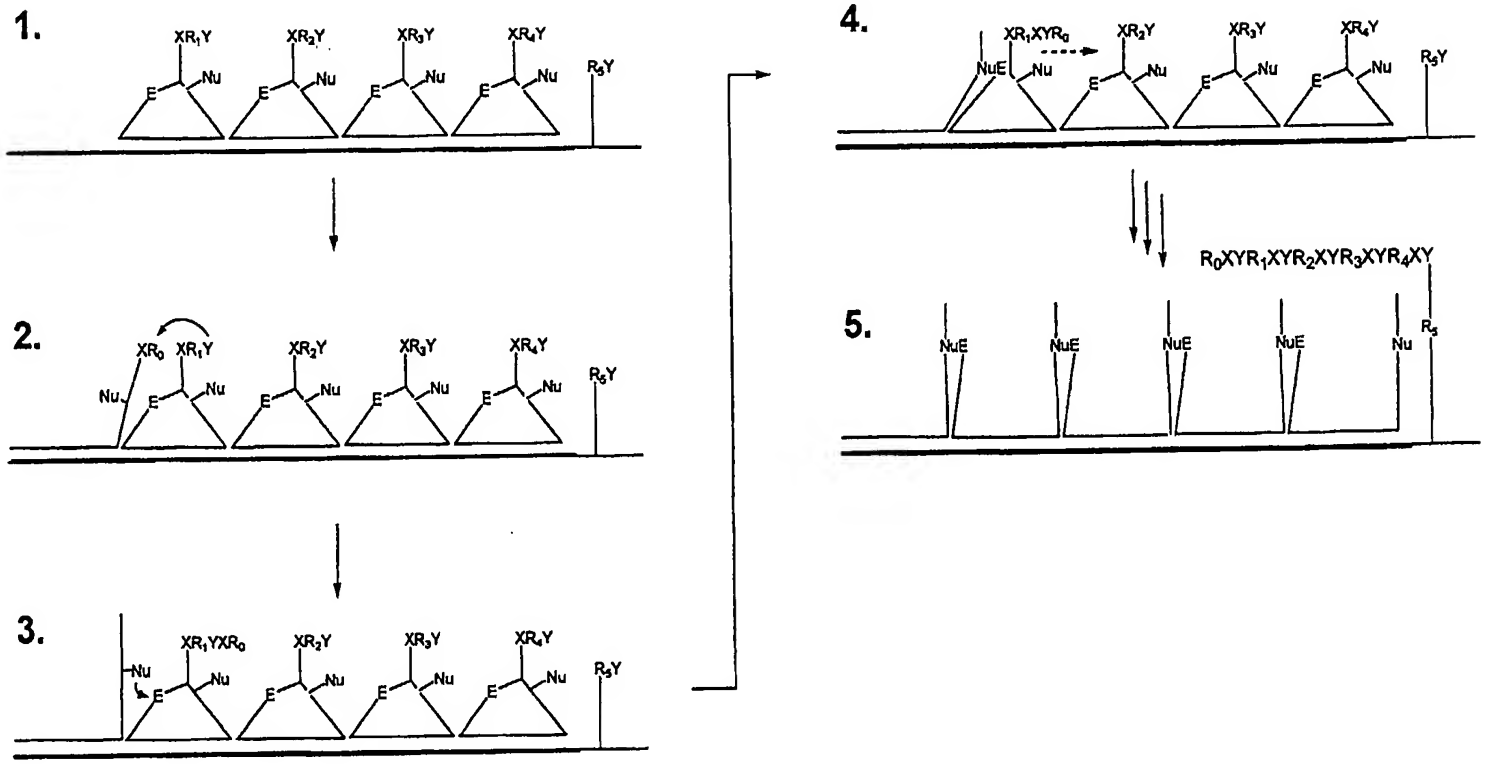
Z = O, S

n = 0, 1

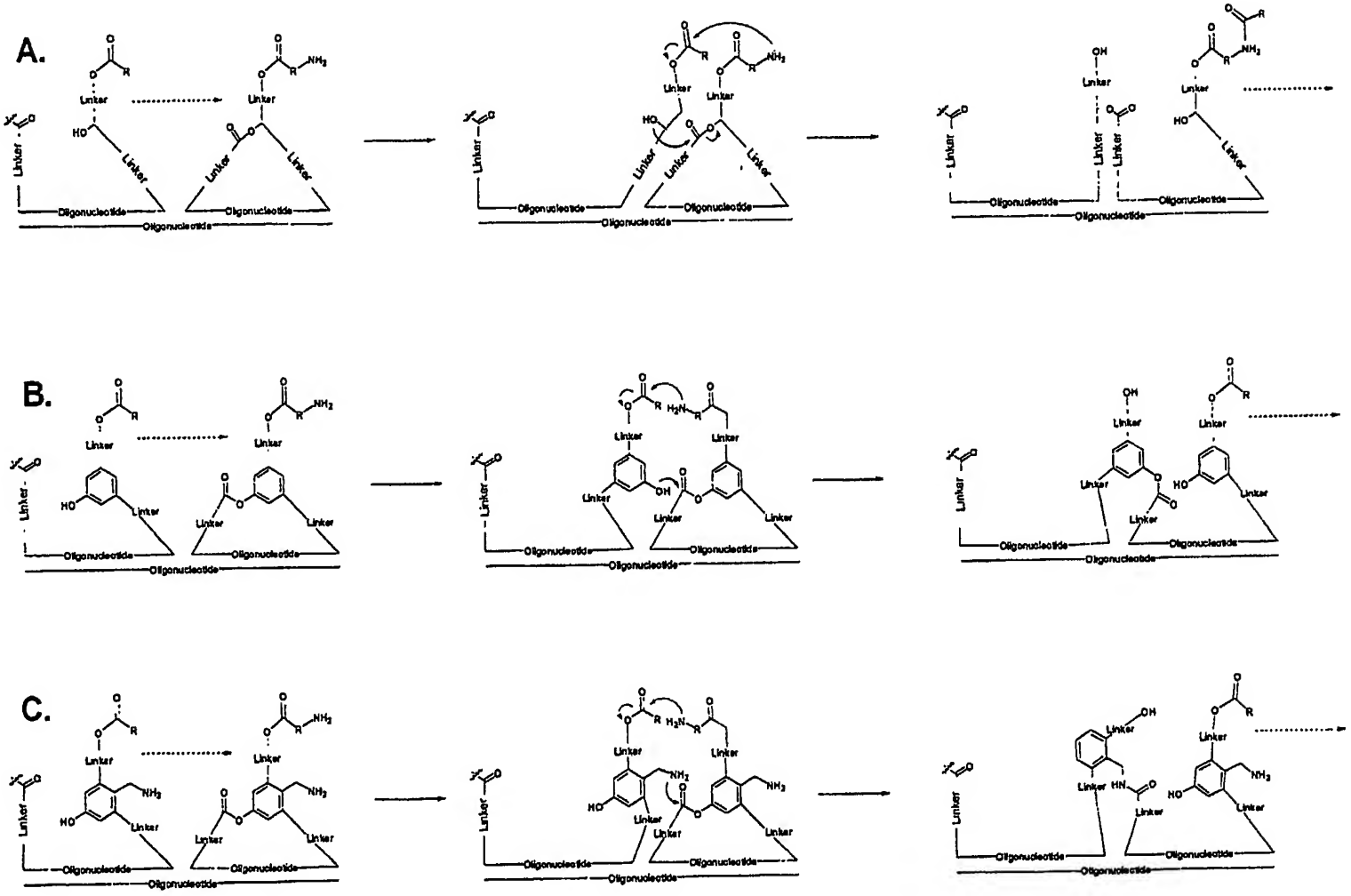
45. Directional encoding by nucleation of hybridization and release



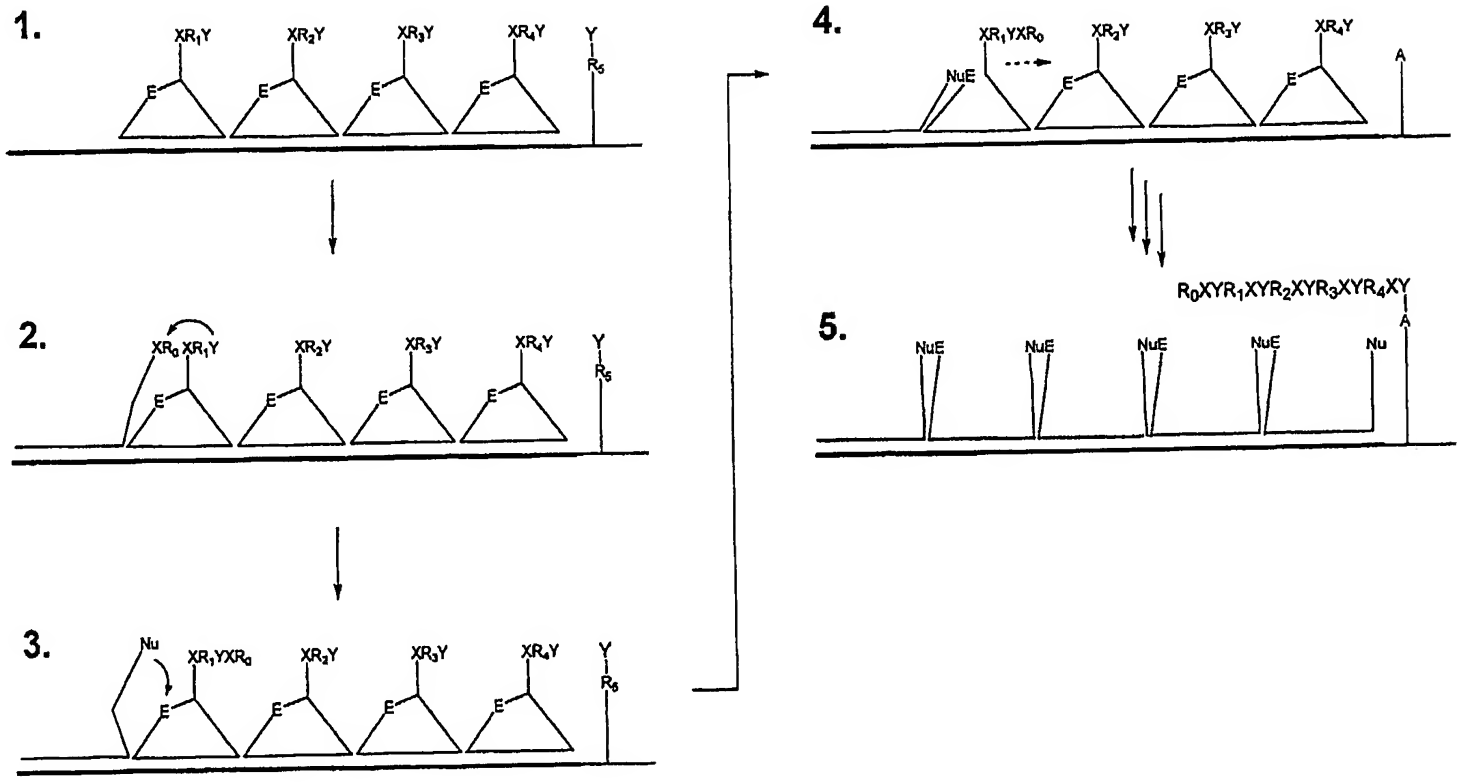
46. Directional encoding by nucleophilic attack and release



46. Example 1: Directional encoding by nucleophilic attack and release

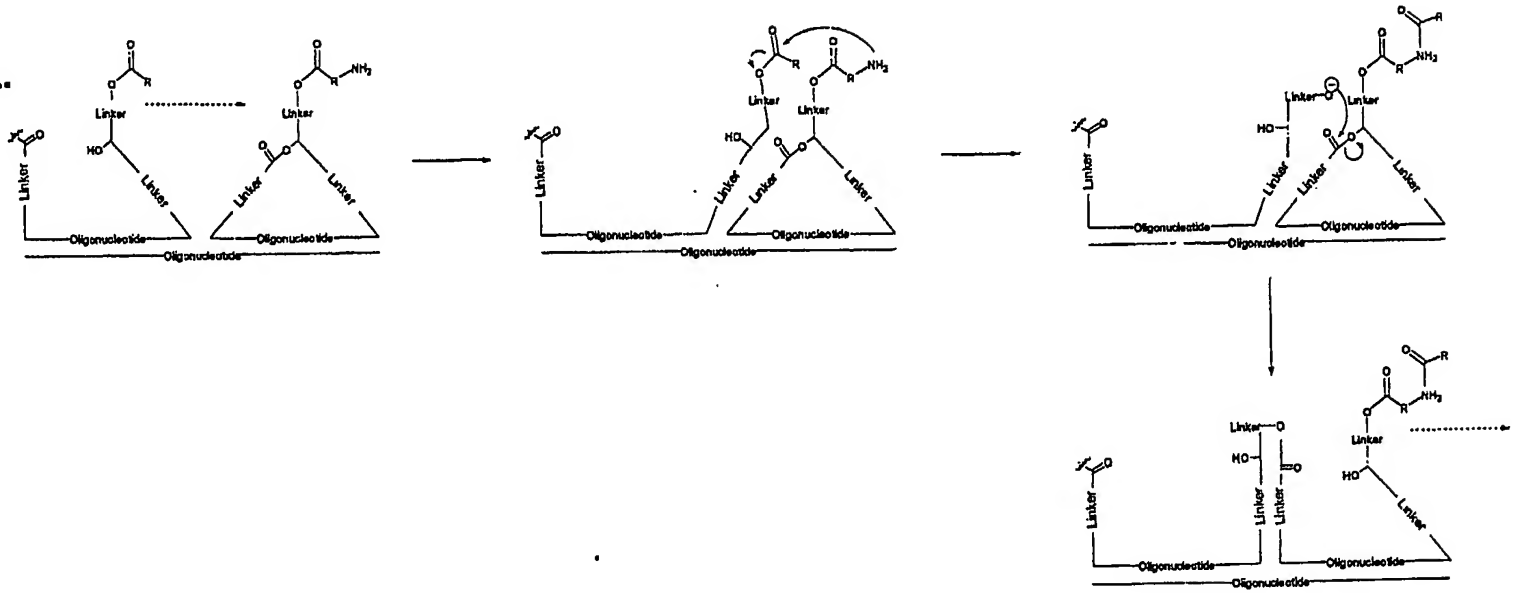


47. Directional encoding by release mediated by induced nucleophilic attack

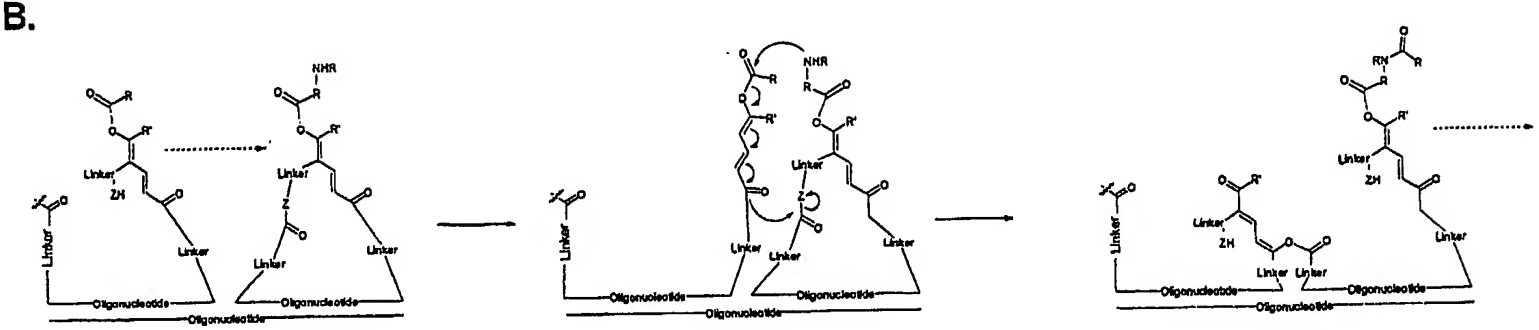


47. Example 1: Directional encoding by induced nucleophilic attack and release

A.



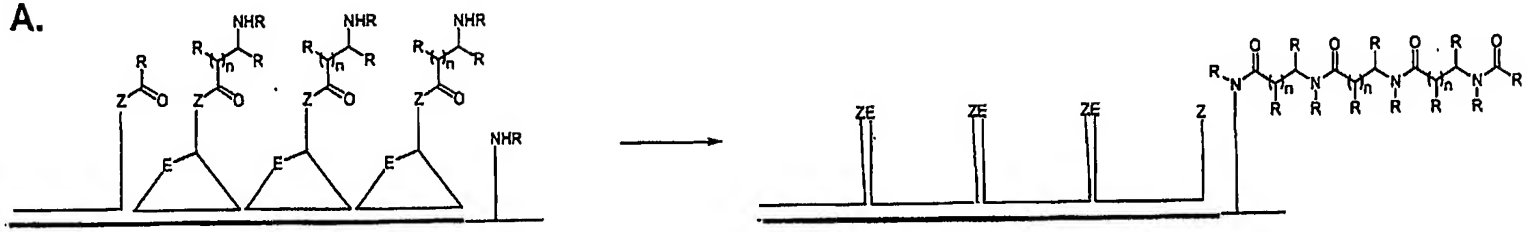
B.



Z = O, S

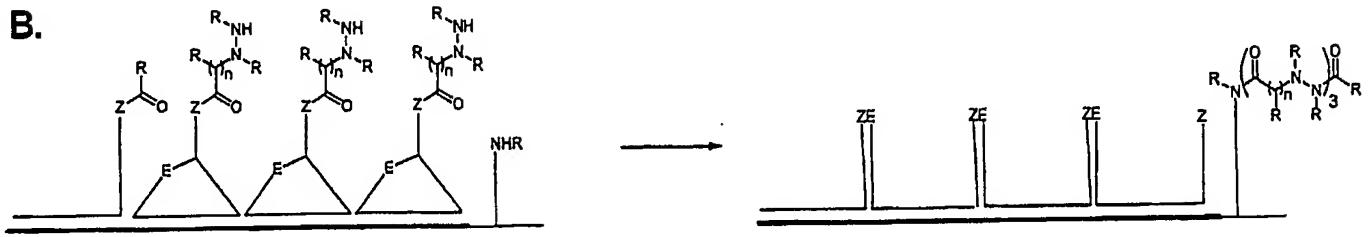
47. Examples 2: Directional polymer encoding of peptides (α, β), peptoids or hydrazinopeptides

A.



Z = O, S
n = 0, 1

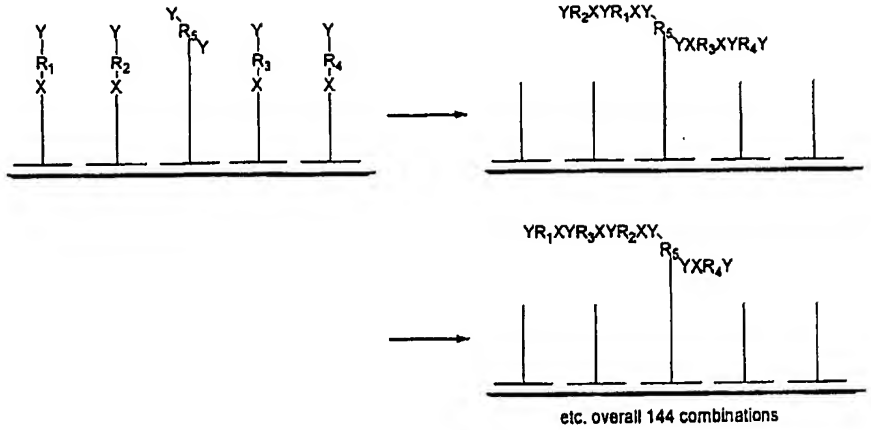
B.



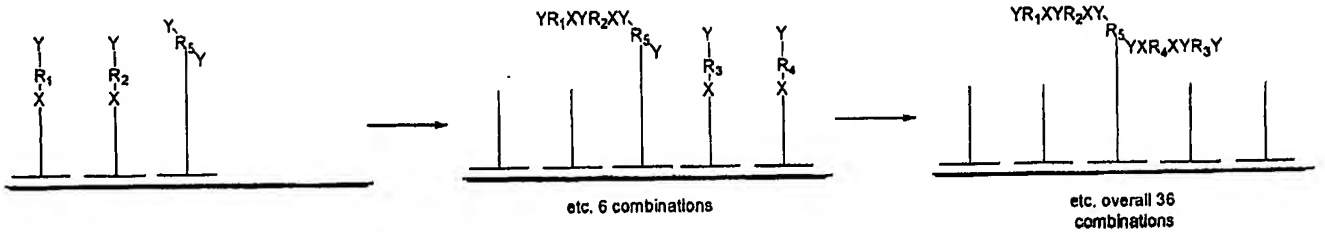
Z = O, S
n = 0, 1

48. Multistep templating

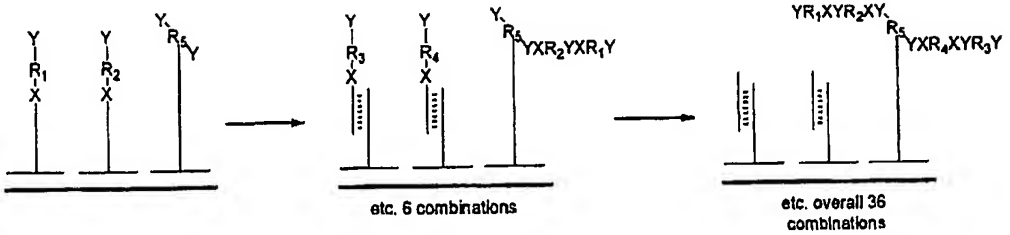
A.



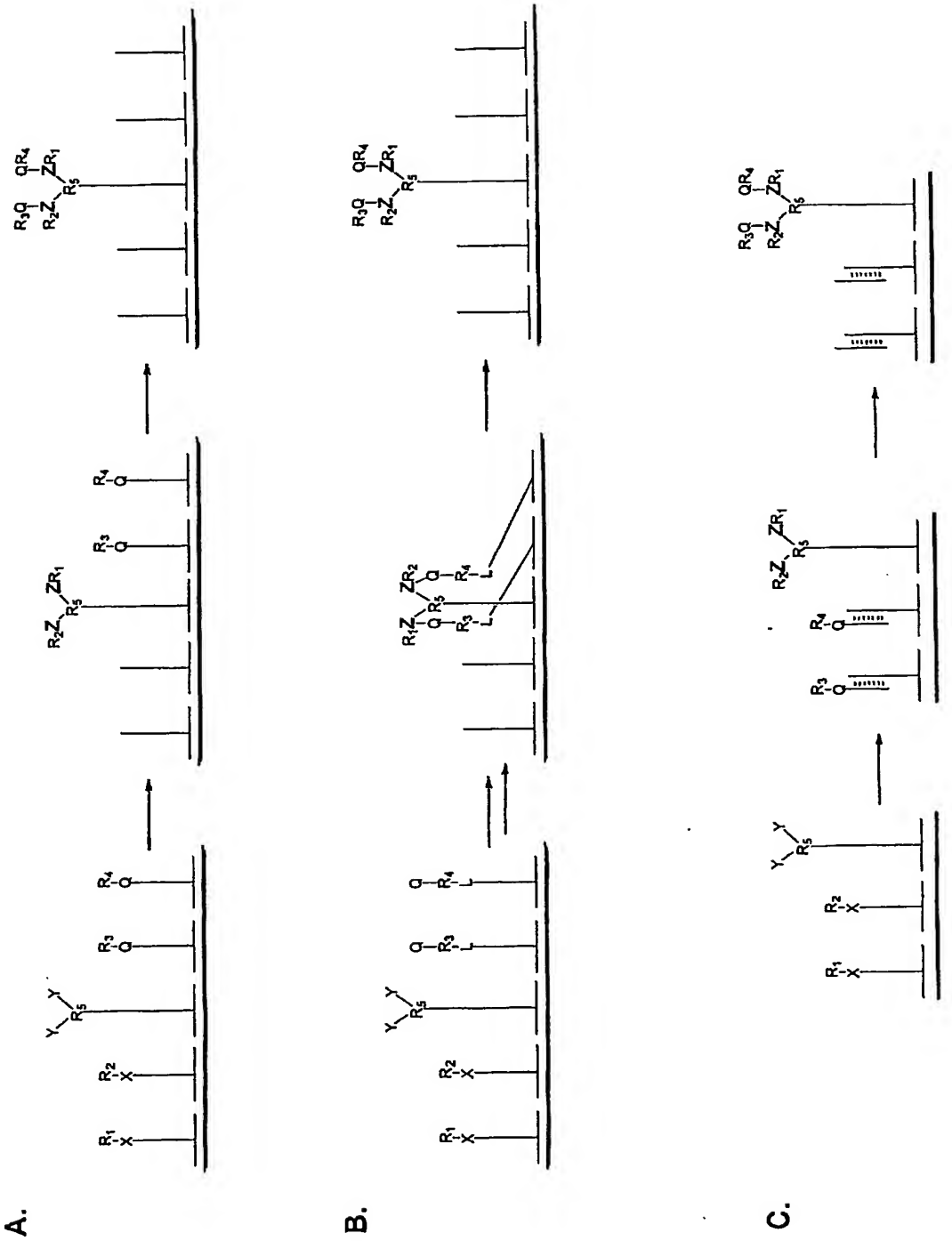
B.



C.



49. Double templating

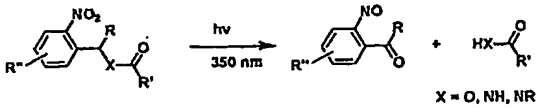


50. Linkers

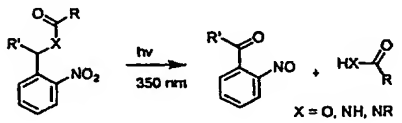
- Carbohydrides and substituted carbohydrides
- Vinyl, polyvinyl and substituted polyvinyl
- Acetylene, polyacetylene
- Aryl/hetaryl, polyaryl/hetaryl and substituted polyaryl/polyhetaryl
- Ethers, polyethers such as e.g. polyethylenglycol and substituted polyethers
- Amines, polyamines and substituted polyamines
- Double stranded, single stranded or partially double stranded natural and unnatural polynucleotides and substituted double stranded, single stranded or partially double stranded natural and unnatural polynucleotides
- Polyamides and natural and unnatural polypeptides and substituted polyamides and natural and unnatural polypeptides.

Figure 51. Cleavable Linkers

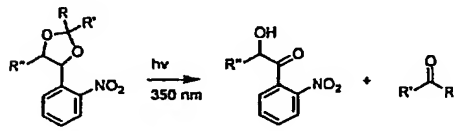
A. Linker for the formation of Ketones, Aldehydes, Amides and Acids



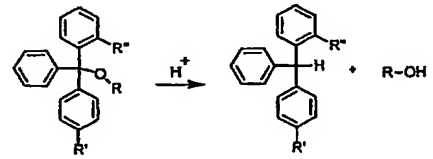
B. Linker for the formation of Ketones, Amides and Acids



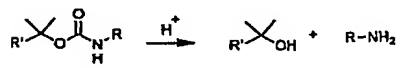
C. Linker for the formation of Aldehydes and Ketones



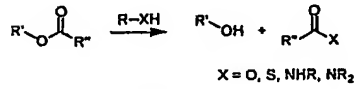
D. Linker for the formation of Alcohols and Acids



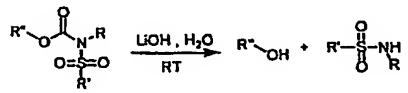
E. Linker for the formation of Amines and Alcohols



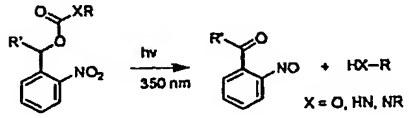
F. Linker for the formation of Esters, Thioesters , Amides and Alcohols



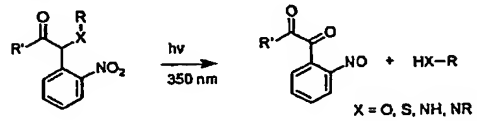
G. Linker for the formation of Sulfonamides and Alcohols



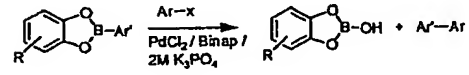
H. Linker for the formation of Ketones, Amines and Alcohols



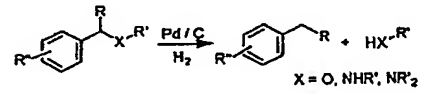
I. Linker for the formation of Ketones, Amines, Alcohols and Mercaptanes



J. Linker for the formation of Biaryl and Bihetaryl

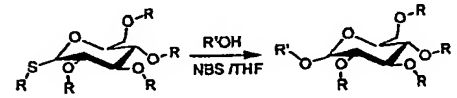


K. Linker for the formation of Benzyles, Amines, Anilins Alcohols and Phenoles

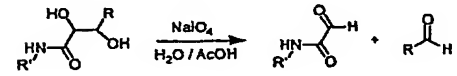


L. Linker for the formation of Mercaptanes

M. Linker for the formation of Glycosides



N. Linker for the formation of Aldehydes and Glyoxylamides



O. Linker for the formation of Aldehydes, Ketones and Aminoalcohols

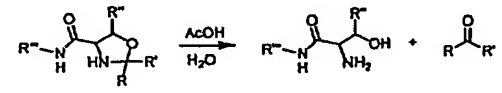


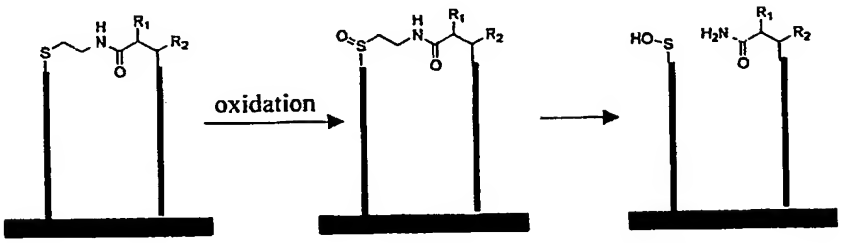
Figure 52. Templated molecules - examples..

1. **Monofunctional, Difunctional, Trifunctional and Oligofunctional open-chain hydrocarbons.**
2. **Monofunctional, Difunctional, Trifunctional and Oligofunctional Nonaromatic Carbocycles.**
   Monocyclic, Bicyclic, Tricyclic and Polycyclic Hydrocarbons
   Bridged Polycyclic Hydrocarbones
3. **Monofunctional, Difunctional, Trifunctional and Oligofunctional Nonaromatic Heterocycles.**
   Monocyclic, Bicyclic, Tricyclic and Polycyclic Heterocycles
   Bridged Polycyclic Heterocycles
4. **Monofunctional, Difunctional, Trifunctional and Oligofunctional Aromatic Carbocycles.**
   Monocyclic, Bicyclic, Tricyclic and Polycyclic Aromatic Carbocycles
5. **Monofunctional, Difunctional, Trifunctional and Oligofunctional Aromatic Heterocycles.**
   Monocyclic, Bicyclic, Tricyclic and Polycyclic Heterocycles
6. **Chelates**
7. **Fullerenes.**
8. **Any combination of the above.**

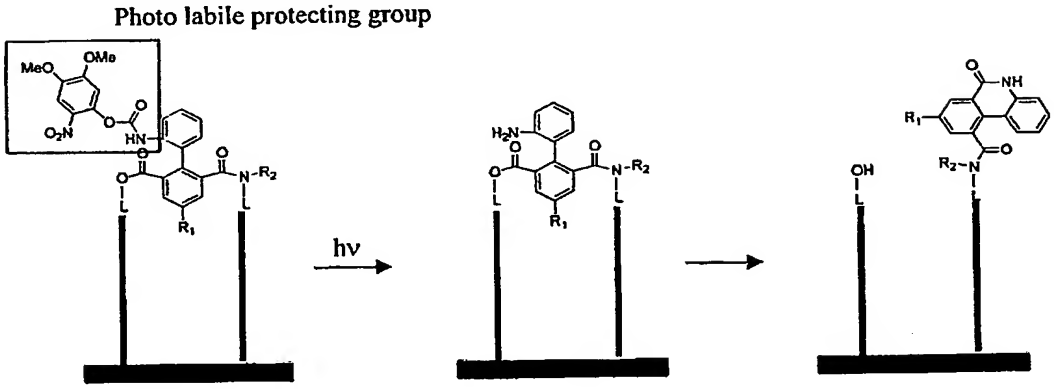
Figure 53. Post-templating modification oftemplated molecule

A Rearrangement and cleavage in one step, eg:

A1

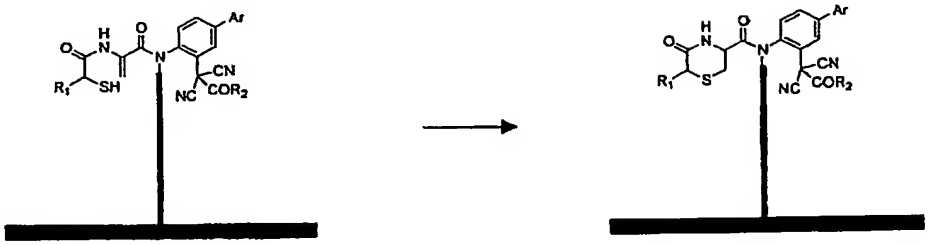


A2

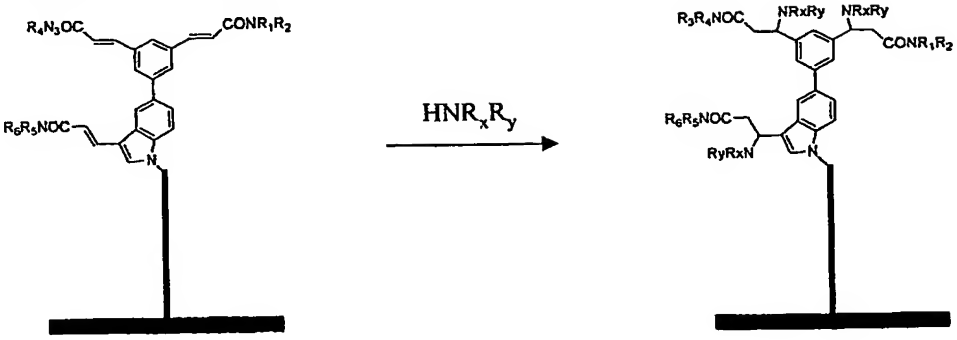


B Reaction of functional groups present in a templated molecule

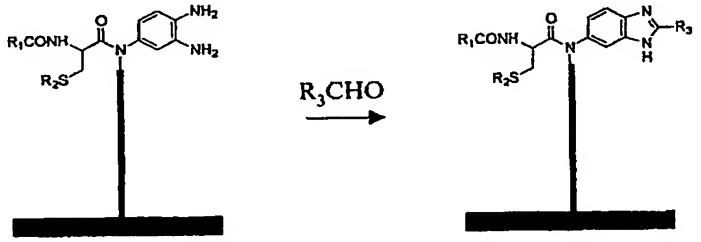
B1 Intramolecular Michael addition:



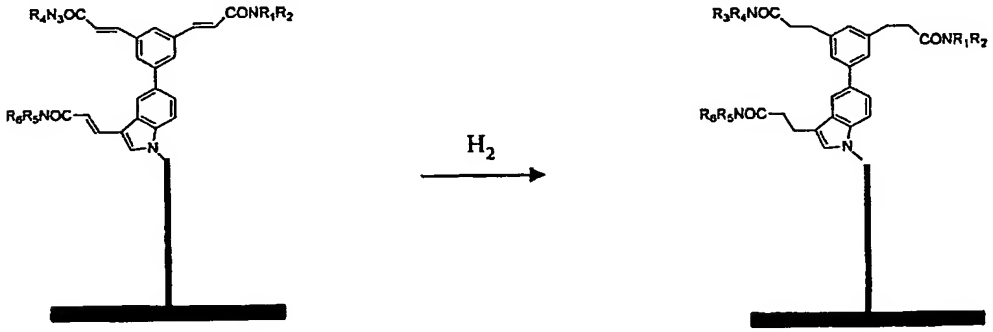
B2 Intermolecular Michael addition:



B3 Reaction of phenylenediamines and aldehydes to form benzimidazoles:



B4 Reduction of multiple bonds:



C Post templating modification of linker to extend the spacing between the template and the templated molecule.